In Vitro Germination of Three Olive Cultivars

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Abstract

Olive seedlings are used in breeding programs as well as rootstocks for grafting of hard–to–root cultivars. However, olive seed germination is not easy, low and slow germination rate are some of the persisted problems. In vitro germination of isolated embryos could be a useful method for increasing the efficiency of germination. One of the main following objectives in embryo culture is the isolation of embryo from the endosperm that contains inhibitory germination substances. This results to shortening of reproductive cycle and the necessary time for obtaining seedlings. In this study, the embryo germination potential of three olive cultivars including 'Manzanilla' and two Iranian cultivars i.e. 'Dezful' and 'Zard' was evaluated. Also the effect of three harvesting times (middle of August, September and October) on growth and development of embryos was studied. Aseptically isolated embryos were cultured in MS medium with one third of macro elements and placed in growth chamber at 25°C day, 21°C night with 16-h photoperiod. According to results, some differences were revealed between germination percent of embryos and the following growth of them. The highest germination percent and seedling formation was obtained at the first culture time with means of 86 and 73 percent, respectively. 'Dezful' cultivar with 90% germination and 79% seedling formation showed the highest values.

INTRODUCTION

The low level of fruit set (1-4%) both in naturally or artificial pollination is one of the limitation in olive breeding (Acebeco et al., 1997). Furthermore, all the following stages, i.e. seed germination, juvenile growth period and transition to the reproductive stage until fruitful inflorescence development, are long and not always synchronous in the seedling population (Rugini, 1986; Acebedo et al., 1997). Early harvesting of not fully ripe fruits was found both to increase the germination percent and shorten the time of germination. However even under such condition the germination rate for many cultivars remained slow and at a low level (Lavee, 1990; Lavee et al., 1996).

It has been demonstrated that the excised olive embryo is capable of in vitro germination prior to full maturation and acquisition of dormancy (Istanbouli and Neville, 1977; Lagarda et al., 1983; Rugini, 1990; Voyiatzis and Pritsa, 1994). Furthermore, the in vitro germination rate appears to be related to embryo development (Lagarda et al., 1983; Voyiatzis and Pritsa, 1994). The main purpose of embryo culture is the shorten the breeding cycle or make possible the seedling material as an appropriate source for isolating viable protoplasts (Rugini and Fedeli, 1990; Canas et al., 1992; Acebedo et al., 1997).

MATERIALS AND METHODS

In order to investigate the effects of harvesting time on the growth and development of isolated embryos, three harvesting times (middles of August, September and October) were compared. The plant materials fruit from 'Manzanilla' and two Iranian cultivars i.e. 'Dezful' and 'Zard' were collected from Roudbar olive research center, the mesocarps were removed and the stony endocarps broken in order to remove the seeds. Seeds were surface sterilized by shaking for 4 min in 0.1% $HgCl_2$, After thorough rinsing

with sterile distilled water the seeds were placed on a double layer of sterile filter papers inside Petri dishes, and 5 ml of sterile distilled water was added and placed overnight at 25°C in the darkness to promote swelling and facilitating the extraction of the embryos. Embryo isolation was performed under sterile conditions by cutting off two lateral sections of the endosperm and releasing the embryo. Embryos were placed in sterile jars (5 per Jar) containing 35 ml of 1/3 strength MS medium (Murashige and Skoog, 1962) with 6% agar, without any plant growth regulators. Jars were placed in a growth chamber at 25°C day, 21°C night with 16-h photoperiod. This experiment was conducted as completely randomized design with 6 replications.

RESULTS AND DISCUSSION

Embryos showed the first signs of germination after four to five days of culture. Cotyledons started to diverge followed by greening and elongation of rootlet. No significant difference in the time of germination was found between cultivars but some differences were observed among three harvesting times in each cultivar. The highest germination percent of 'Dezful' and 'Manzanilla' cultivars was obtained at the middle of August and germination reduced at the second and third harvesting times, but embryos of cv. 'Zard' showed reduction of germination percent only at the third harvesting time (Table 1).

The next stages of germinated embryos growth and developments showed positive relation between seedling formation percent and seed germination in all tested cultivars. The highest values were obtained at the first harvesting time. Seedlings quality as compared by shoot length showed some differences among cultivars, and 'Dezful', 'Manzanilla' and 'Zard' cultivars ranked from first to third, respectively (Table 1).

Due to differences between growth and development phases within cultivars, the germination responses of embryos could be different (Linan et al., 1999). However, in this experiment cultivars showed similar response of seed germination percent and seedling formation percent in each harvesting time, therefore it can be concluded that embryo growth phases of these three cultivars were approximately similar. The lower germination and seedling formation at the third harvesting time can be attributed to occurring of dormancy in embryos, as embryo internal factors can influence its dormancy (Voyiatzis, 1995). Increasing of embryos germination after two weeks of cold treatment at 10°C is a confirmation for this conclusion (Pritsa and Voyiatzis, 1999).

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Tables

Table 1. In vitro germination and seedling formation of three olive cultivars at three planting times.

Cultivar						
Manzanilla		Dezful		Zard		
Seedling (%)	Germ (%.)	Seedling (%)	Germ (%.)	Seedling (%)	Germ. (%)	Harvesting Time
70.2	85.5	79	90	70.1	85	Middle of August
66	85.5	51.1	76	45	67	Middle of September
30.7	42.8	26.5	34.8	40.9	57	Middle of October