Establishment and optimization of micrografting assays with almond (*Prunus dulcis*) Portuguese varieties

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INTRODUCTION

In the last years, the **almonds culture** has increased in **Portugal** with an introduction of foreign cultivars neglecting the **traditional varieties**, known for the high quality of the fruits [1].

Micrografting consists in the placement of the scion onto a decapitated rootstock in aseptic conditions [2]. The compatibility between the scion and rootstock, the culture medium, the grafting type, the natural organic compounds and growth regulators influenced the growth rate and success of the micrograft [3].

In order to establish a **protocol for micrografting assays with almond Portuguese varieties**, slit micrografting technique was evaluated in Rabo de Zorra, Gama Dura and Canhota varieties. The effect of plant growth regulators (BAP and IBA) and activated charcoal on culture medium were also evaluated during micrografting assays. The effect of auxin IBA on root induction was also analyzed during rootstock assays.

METHODOLOGY

Establishment of in vitro cultures for scion and rootstock

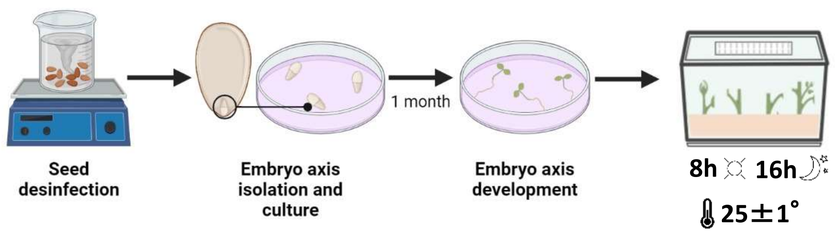

Figure 1. Mature kernels were surface sterilized; the seed coats were removed, and cultured in a petri dish lined with sterile paper moistened with a 1 mg/L GA_3; in vitro seedlings were decapitated above the cotyledons and cultured in MS medium + 30 g/L sucrose + 1 mg/L BAP + 7 g/L agar, and subculture every 3 weeks to the same medium.

Rooting assays

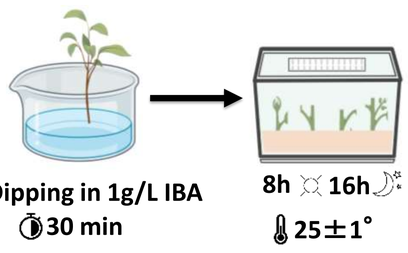

Figure 2. Explants from Canhota and bitter almond were dipping 30 minutes in a solution of 1 g/L IBA and transferred to MS + 30 g/L sucrose + 7 g/L agar. The rooting rate and the number of roots was registered after 2 months.

Micrografting assays

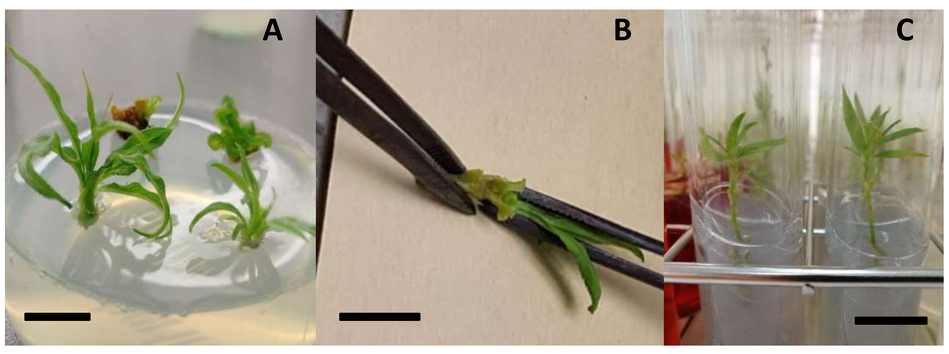

Figure 3. Micrografting of *Prunus dulcis*. Establishment and multiplication of rootstock (A); slit micrografting (B); micrografts in MS medium (C). Scale bar: 1cm. The micrografts were cultured in MS medium + 30 g/L sucrose + 7 g/L agar (M1 medium); M1 medium + 1 mg/L BAP; M1 medium + 2 g/L AC. The micrografting success were evaluated after 2 months.

RESULTS

Rooting assays

IBA dipping: Higher formation of roots in bitter almond but not in Canhota, after two months of culture;

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Table 1. Effect of IBA dipping procedure in the formation of roots.

	Rooting rate (%)	Number of roots
Bitter almond	60	13
Canhota	6.6	1

Micrografting assays

The use of PGR and AC free MS medium contribute to the success of micrografting. The bitter almond x canhota combination presented a higher micrograft lengh, and similar healing and success rates. The use of IBA seems to improve the micrograft growth, the healing rate and the rooting rate.

Table 2. Effect of final medium in micrograft length, healing rate, success rate and shoot formation in the rootstock in micrografting.

MS+BAP					
Rootstock x scion	**N**	**Micrograft length (cm)**	**Healing (%)**	**Micrograft Success (%)**	**Shoot formation in the rootstock (%)**
Bitter almond x Canhota	4	2.75	100	100	100
R. Zorra x R. Zorra	5	1.42	80	40	40
		2.01±1.2a	88.9	62.5	66.67
MS+AC					
Rootstock x scion	**N**	**Micrograft length (cm)**	**Healing (%)**	**Micrograft success (%)**	**Shoot formation in the rootstock (%)**
Bitter Almond x Canhota	8	3.3	75	75	75
R. Zorra x R. Zorra	6	2.08	100	33.33	83.3
Canhota x Canhota	7	1.98	100	85.7	57.14
G. Dura x G. Dura	10	1.75	100	30	30
		2.26±1.5a	87.1	45.16	58.06
MS					
Rootstock x scion	**N**	**Micrograft length (cm)**	**Healing (%)**	**Micrograft success (%)**	**Shoot formation in the rootstock (%)**
Bitter Almond x Canhota	10	2.18	80	70	20
R. Zorra x R. Zorra	9	1.75	88.89	88.89	77.78
Canhota x Canhota	6	1.8	100	16.67	66.67
G. Dura x G. Dura	8	1.84	100	100	62.5
		1.91±0.4a	90.9	72.72	54.54

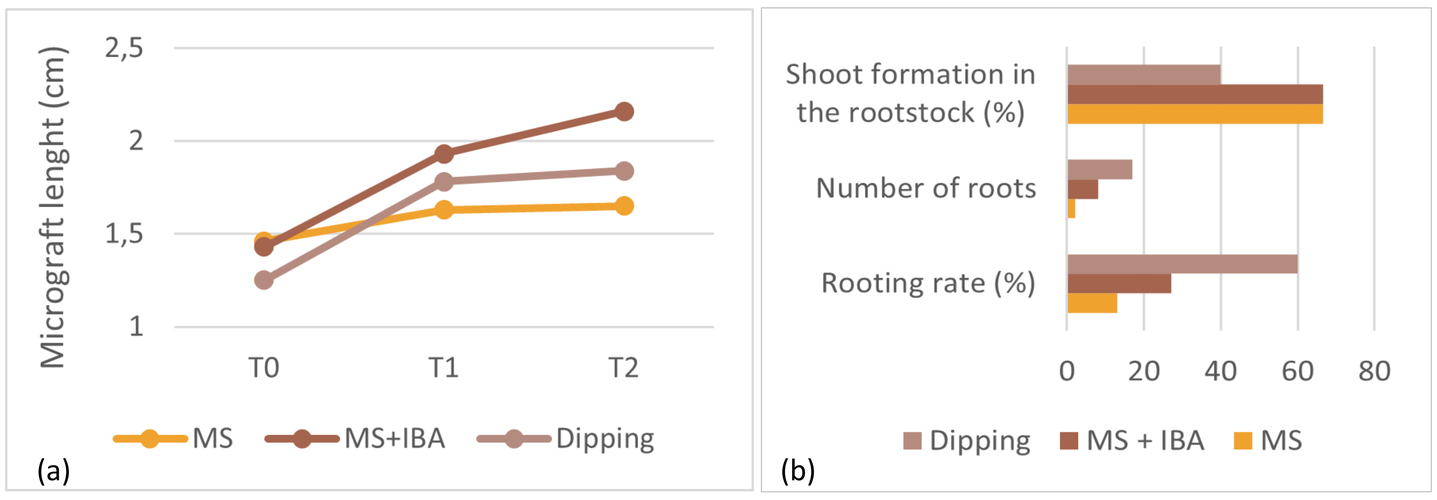

Figure 4. Effect of auxin IBA on micrografting. (a) Micrograft length evaluation after (T0), 1 month (T1) and 2 months (T2) of micrograft; (b) Shoot formation in the bitter almond rootstock (%), number of roots and rooting rate (%), after 2 months of micrografts. Significantly differences were registered between MS and MS+IBA on shoot length after 2 months (T2), at p≤0.05, using Kruskal-Wallis test.

A success rate of micrografting around 73% was achieved with PGR free MS medium. The results reinforcing the good compatibility observed between Bitter almond rootstock and Canhota variety. **IBA influences the root formation, and the dip-quick approach on rootstock contributes to the growth of the scions**. New experiments to test other concentrations and/or others PGR will be performed in these varieties. This work represents a step forward in the field of multiplication of traditional almond varieties disease-free, which has been overlooked in the news almond orchards.

References:
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[3] Pahnekolayi, M.D.; Tehranifar, A.; Samiei, L.; Shoor, M. Optimizing culture medium ingredients and micrografting devices can promote in vitro micrografting of cut roses on different rootstocks. *Plant Cell Tiss Organ Culture*. **2019**, *137*, 265-274.