OLIVE EMBRYO DEVELOPMENT STAGE AND THE POSSIBILITY OF OBTAINING VIABLE SEEDLINGS

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Abstract

When 'Manzanillo' olive embryos were excised at successive dates and cultured on one-third strength Murashige-Skoog medium, three stages of differing growth potential were observed: 1) An initial stage in which no germination was obtained, 2) An intermediate 'critical' period in which germination and viability percentage increased according to date and 3) A final stage, from the time when the embryo reached full size, in which germination and viability were maximum. In the intermediate 'critical' period the seed had obtained its final length and the embryo was undergoing rapid expansive growth. Anatomical studies indicated the presence of the future root and shoot apices and well-defined provascular strands throughout this period. As time progressed, however, the embryo tissues seemed to acquire greater metabolic content and cytoplasmic integrity.

1. Introduction

Among the benefits of obtaining seedlings from excised developing embryos are the shortening of the reproductive cycle and the time necessary to obtain seedlings, the elimination of unnecessary maturation time and the circumvention of dormancy factors (Monnier, 1995). The reduction of these limitations in the olive in order to obtain viable seedlings more rapidly is highly desirable for olive breeding programs (Acebedo *et al.*, 1997). It has been demonstrated that the excised olive embryo is capable of *in vitro* germination prior to full maturation and the acquisition of dormancy (Lagarda *et al.*, 1983; Rugini, 1990; Voyiatzis and Pritsa, 1994; Acebedo *et al.*, 1997). Furthermore the *in vitro* germination rate appears to be related to embryo development (Lagarda *et al.*, 1983; Voyiatzis and Pritsa, 1994).

The objectives of this study were to germinate excised olive embryos *in vitro* and to determine the embryo developmental stage and characteristics necessary for obtaining viable seedlings.

2. Materials and methods

The plant material used was from 'Manzanilla de Sevilla' grown in Sevilla, Spain. Fruits were harvested at weekly intervals, the mesocarp (flesh) was removed and the stony endocarp was broken in order to remove the seed (Crisosto and Sutter, 1985; Sotomayor-León and Caballero, 1990). The seeds were sterilized with hypochlorite and the embryos excised under sterile conditions and planted individually in sealed sterile test tubes containing 10 ml of culture medium (Acebedo, 1997). For the earliest dates, when the embryo was too small to isolate, the whole, sterilized seed was planted. A 1/3 strength MS medium (Murashige and Skoog, 1962) was used. The culture tubes were placed in a growth chamber at $25 \pm 2^\circ\text{C}$ with a 16-h photoperiod $111 \text{ mE m}^{-2} \text{ s}^{-1}$.

Germination was assessed as elongation and opening of the root-hypocotyl axis and as greening of the cotyledons. Seedling formation was assessed as tap root development and elongation, shoot development and true leaf formation.

For 1993 and 1994 embryos were excised and cultured at weekly intervals from 3 to 14 weeks after bloom. In 1995 embryos were excised and cultured based on both date (5 consecutive weeks June 21 -July 19) and size (4-5.5 , 5.6-7.5, 7.6-9.5 and >9.5 mm embryo length) and anatomical studies were performed to evaluate embryo development. For the anatomical studies standard paraffin techniques for optical microscopy (Jensen, 1962) were utilized and the sections were stained with safranin, crystal violet and light green (Gerlach, 1969).

3. Results

Embryo germination *in vitro* was significant from 12 weeks after flowering in 1993 (table 1) and 13 weeks after flowering in 1994 (table 2). At these times the embryos had achieved 6 mm length. Seedling formation, the continued growth after germination to form a viable seedling, was substantially lower than germination at the earlier dates and then increased (tables 1 and 2). When embryos were tested taking into account both age and size, both of these factors had a favorable influence on *in vitro* germination (table 3).

The anatomical studies revealed that at all dates on which germination was achieved basic tissues which might enhance germination and future survival were present. These tissues were the provascular tissue, the shoot apex and the root apex. The staining of the cytoplasm and cellular contents of the older and larger embryos, however, was much more intense and complex than that observed in the younger and smaller embryos. Additionally, the cells of the older and larger embryos appeared fuller and less wrinkled.

4. Conclusions

In vitro germination of olive embryos depends on both date and size (table 1, 2, 3). These results support those of previous studies (Lagarda *et al.*, 1983; Rugini, 1990; Voyiatzis and Pritsa, 1994). While obtaining viable seedlings depends on the same factors, the viable seedling success rate tends to be lower than that for germination, especially at the earlier dates (table 1, 2). The characteristics associated with obtaining a high level of viable seedlings may be summarized as: The fruit and seed have reached final size; the pit (endocarp) has hardened, the endosperm has hardened, and the embryo has formed its mature structure consisting of cotyledons and root-hypocotyl axis and reached a minimum length of 6 mm.

Anatomically the capacity of developing olive embryos to germinate *in vitro* seems to be related to a greater metabolic content and cytoplasmic integrity. The formation of anatomical structures such as provascular tissues, shoot apex and root apex occurred at all dates examined and did not seem to limit the germination capacity.

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Table 1. *In vitro* germination and seedling formation obtained from olive embryos at different times after bloom. (1993).

Sample Date	weeks after bloom	Fruit diameter (mm)	Seed length (mm)	Embryo length (mm)	Germin. <i>In vitro</i> (%)	Seedling formn. (%)
12 May	3	3	0.7	-	0	0
19 May	4	3.7	1.8	-	0	0
26 May	5	4.8	2.9	-	0	0
2 June	6	6.4	5.3	-	0	0
16 June	8	9.0	7.7	-	0	0
21 June	9	9.8	8.0	-	0	0
29 June	10	12	9.2	-	0	0
7 July	11	12	9.2	3.5	0	0
13 July	12	12	9.2	6.8	62	25
22 July	13	12	9.2	7.9	80	80
5 Aug.	15	13	9.3	8.1	80	80

Table 2. *In vitro* germination and seedling formation obtained from olive embryos at different times after bloom. (1994).

Sample Date	weeks after bloom	Fruit diameter (mm)	Seed length (mm)	Embryo length (mm)	Germin. <i>In vitro</i> (%)	Seedling formn. (%)
20 May	4	5.0	3.4	-	0	0
26 May	5	6.8	4.3	-	0	0
8 June	7	9.6	8.0	-	0	0
15 June	8	10.9	8.0	-	0	0
29 June	10	11.2	8.8	1.0-2.0	0	0
5 July	11	-	8.8	3.0	0	0
12 July	12	-	8.8	4.5	0	0
19 July	13	-	8.8	6.0	85	56
26 July	14	-	8.8	6.5	90	65
2 Aug.	15	-	8.8	7.0	100	75

Table 3. Germination and seedling formation from excised olive embryos of different age and size. (1995).

Date	Embryo size (length, mm)							
	A (4-5.5)		B (5.6-7.5)		C (7.6-9.5)		D (>9.5)	
	Germ. (%)	Seedling (%)	Germ. (%)	Seedling (%)	Germ. (%)	Seedling (%)	Germ. (%)	Seedling (%)
21-6	4	0	45	23	-	-	-	-
28-6	25.4	17.6	48	24	72	50	-	-
5-7	56.5	24	65	30	80	50	-	-
12-7	-	-	83	74	83	83	83	83
19-7	-	-	80	83	81	80	75	75