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# The Influence of Naa on Embryo Germination of the Olive

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**ABSTRACT**

Embryo germination was experimented over a period of three years as part of the embryogenesis of the olive endocarps. During April the endocarps were treated with immersion of NAA, $C_{12}H_{10}O_2$, per 12 hours in three different solutions, 50, 100, 500 ppm. The endocarps were stratified in a substrate sand+peat+endocarps (3:1:1), in a habitat with temperature from 15⁰C to 17⁰C for wood decomposition and embryonic awakening. After planting and germination the results were as follows: n0 zygotic embryos, nopolyembryos, no somatic embryos, nº pseudo- embryos. The results proved NAA100 and NAA50 for better embryogenesis, respectively: NAA100/KAN 17.6% and NAA100/HH 73,9%. 93% of germination force derives from cellular growth within the zygote. KAN cv had exaggerated pseudoembryos 83-86%, whereas HH cv 29-40%. The concentrations from 50 to 100 ppm, yielded zygotic embryo 43%, Polyembryos 1.5%, somatic embryos 0.75%. Concentration 500 ppm developed much more polyembryony. High percentage of germination occurred only when endocarp structure was changed, thus lowering its resistance to embryo spreading. NAA served as a good promoter of the embryogenesis process and physiological ripeness. Under these circumstances the effect of hormone induction is 9.5% and 30%.

**KEYWORDS** Diabetic Ketoacidosis, Regular Insulin, Aspart

**Introduction**

Olive endocarps are the initial material needed for the vegetative method of propagation with grafting (Bartolini et al., 1989; Bellini., 1993). Although they have different morphological characteristics, the process of embryogenesis and capacity of germination display considerable variability (Bellini et al., 2004 ; Ismaili et al., 2012). Several in vitro research treat the use of some regulators as promoters of embryo germination, which served as objects of experimentation as of their importance (imaili et al., 2014). Several studies on morphogenesis and rhinogenesis are important as their results influence on the improvement of costs, despite the difficulties ran into while implemented on the olive (Bartolini et al., 1989). Sexual propagation presents some difficulties of the biological character (Bellini et al., 2004), thus physiological ripeness and embryo germination need to identify primarily the ability and energy of embryonic germination (Engelmann et al., 2011). On the other hand other ways that improve this phenomenon via auxin induction must be identified (Ismaili., 2014). Different research consider of great importance the origin of endocarps, physiological ripeness as well as the interrelation among physiological processes of sleep, awakening and germination (Mehri et al., 2001). This experiment has analysed exactly the influence of NAA on induction, physiological ripeness or embryogenesis, meristem regeneration and its influence on embryo germination.

**Material and Methods**

The endocarps were extracted during the period of ripeness. The endocarps were cleaned by the fats in immersion of soda 3% and temperature 35-40⁰C (NaOH), the seeds were dried and preserved at parchment packets. During April the endocarps were pre-treated with stimulation per 12 hours in NAA acid solution of concentration 50, 100, 500 (ppm). Naphthaleneacetic acid is a chemical solution ($C_{12}H_{10}O_2$). 1-Naphthaleneacetic acid (NAA) is an organic compound made up of maternal solution (ethanol + NAA = maternal solution). The solution for immersion contains 2,5% ethanol and 97.5 distilled $H_20$. The endocarps were later stratified in a substrate sand+ peat +endocarp (3:1:1), in a habitat with temperature 15-17⁰C per 35days. When the shell started cracking and the embryos were revealed, planting was performed in polyethylene strata in pans with 50 seeds/repetition in a substrate river sand + peat (1:1), in a polyethylene tunnel. Thermal regimes were: 18⁰C during the night and 27⁰C during the day.

**Experimental scheme**: (i) KAN/NAA50 ppm, (ii) KAN/NAA100ppm, (iii) KAN/NAA500 ppm (iv) KAN/CONTROL ($H_2O$) (v) HH/NAA50 ppm, (vi) HH/NAA100 ppm, (vii) HH/ NAA500ppm (viii) HH/CONTROL ($H_2O$). Each treatment (3 x100) endocarps. **Research indices:** (i) 50 embryos per each treatment included the analysis of: n⁰ zygotic embryos, nºpolyembryos, nºsomatic embryos, nº pseudoembryos. **Statistical analysis;** Jmp software, descriptive analysis per variance and physiological correlations at a significant level of ($p<0.05$) (SAS JMP. 2008).

**Results and Discussion**

**Variance analysis**, proved the variability through treatments caused by the phytoregulator per n⁰ zygotic embryos, nº polyembryos, nº somatic embryos and pseudoembryony. The endocarps have undergone physiological ripeness after harvesting up to embryo germination. This process increased the albumen and carbon hydrates necessary for the embryogenesis stages as well as germination as e potential cellular zygote. Table 1 and table 2, NAA 50 and 100 ppm had favourable embryogenesis and germination. Germination results were of genetic character, as the genotypes themselves had different characteristics and results, t*ukey-cramer, p=0.05*, associated with e high coefficient of variation, cv= 21.6%. NAA 50 and NAA100 differentiated (13%-73.9%) embryo germination. The highest pseudoembryos in Control starts decreasing until it is treated with NAA50 ppm. (Con>NAA500>NAA100>-NAA50: Pseudoembryos %)

**Table 1.** Analysis of variance for the data of NAA on embryo germination of the olive

| Trait Treatment | NºZygotic embryos | Nº Polyembryos | Somatic embryos | Pseudo-embryos |
|---|---|---|---|---|
| CONTR/KAN | 7.9±0.35 **F** | 0.10±0.10 **E** | 0.00±0.00 **E** | 83.3±1.52 **A** |
| CONTR/HH | 44.0±3.00 **D** | 0.40±0.10 **DE** | 0.20±0.20 **DE** | 40.6±1.52 **B** |
| NAA100/KAN | 17.6±1.68 **E** | 0.86±0.15 **BC** | 0.50±0.20 **CD** | 86.0±1.00 **A** |
| NAA100/HH | 73.9±1.00 **A** | 1.43±0.11 **A** | 1.16±0.11 **AB** | 29.0±2.00 **D** |
| NAA50/KAN | 13.0±1.55 **EF** | 0.56±0.15 **CD** | 0.47±0.11 **D** | 85.0±1.00 **A** |

| Trait Treatment | NºZygotic embryos | Nº Polyembryos | Somatic embryos | Pseudo-embryos |
|---|---|---|---|---|
| NAA50/HH | 67.6±3.05 **B** | 1.40±0.10 **A** | 1.00±0.09 **B** | 30.6±2.08 **CD** |
| NAA500/ KAN | 13.5±1.85 **EF** | 0.76±0.25 **BCD** | 0.84±0.06 **BC** | 86.3±1.52 **A** |
| NAA500/ HH | 56.6±2.51 **C** | 1.13±0.11 **AB** | 1.36±0.05 **A** | 33.3±1.15 **C** |
| Min | 7.9 | 0.10 | 0.00 | 29.0 |
| Max | 73.9 | 1.43 | 1.36 | 86.3 |
| Mean | 36.7 | 0.83 | 0.69 | 59.2 |
| Std dev | 1.87 | 0.53 | 0.10 | 1.47 |
| CV | 21.6 | 63.8 | 14.4 | 2.5 |
| Prob > F | 0.0122* | 0.0169* | 0.0029* | 0.0111* |

KAN-Kaninjot cv, HH-Holli Himares cv Levels not connected by same letter are significantly different

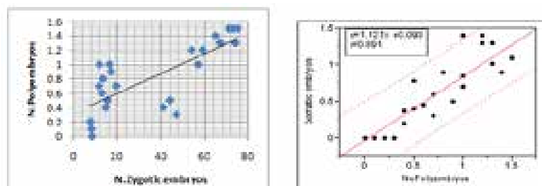

Figure 1 (left) Dendogram for orthogonal regression of nº Zygotic embryos by nº Polyembryos and their probability
Figure 2 (right) Dendogram for orthogonal regression of Somatic embryos By NoPolyembryos and their probability.

**Forms of embryonic induction:** Table-1, presents the embryonic physiological classifications: **(i)** Normal embryos, deriving from the zygote, are heterozygote as they were formed by the pollination of a cell ♀ with a cell ♂ with (**n** chromosomes). After germination they are easily identified as they have a vegetative meristem in the polar axis and a root meristem in the second line (ii) embryos of the type (*polyembryos*) have differentiated several vegetative buds within the same embryo, (iii) **pseudoembryos** have a high percentage of abnormal embryos, which were barren at the beginning, or several embryos which did not germinate or were etiolated, faded or dried as soon as they germinated (Guerra et al., 1998).

**Table 2.** The main data of the analysis of variance on embryo germination

| Source Traits | DF | Sum of Squares | Mean Square | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Nºzygotic embrios | 7 | 15257.716 | 2179.67 | 505.9204 | <.0001* |
| Polyembryos | 7 | 4.7200000 | 0.674286 | 32.3657 | <.0001* |
| Somatic embrios | 7 | 4.8114958 | 0.687357 | 44.6940 | <.0001* |
| Pseudoembrios | 7 | 16323.625 | 2331.95 | 999.4056 | <.0001* |

For the normal embryos referring to total cumulative variance, (*level=0.001**), NAA, generally stimulated favourable results for germination, whereas control had unfavourable results: (*AIA200>AIA500>AIA50>Controlli (% germination)*. The analysis highlighted two physiological correlations: (i) between NAA – % germination, where r2=*0.922* and (ii) between Control – % germination, where $r^2$*=0.422.* Correlation decreased up to Control. Obviously NAA has formulated its role of 92.2%. per embryo germination. Referring to table 1 & dendrogrames 1, two genotypes with the solution NAA100 have dominant embryonic germination respectively 17.6% (KAN) and 73.9% (HH), whereas their Control respectively 7.9%, 44%. Under these had genetic character, as the data between both genotypes had high variation cv= 38.7%. KAN/NAA100= 48.8%, KONTROL/HH=115%, NAA100/HH=200%. NAA improved embryogenesis in correlation with its endogenous capacity, but was not able to transform them. ***Prob > F =*** *0.0111** circumstances the effect of hormone induction is 9.5% and 30%. Germination as well as awakening

**Pseudoembryony or endocarps with poor or missing embryos** resulted in high levels and at a great amplitude (30.6-86%). In this respect Kaninjot had 86% poor embryos and dominated, whereas Himara with 29-40.6% and cv= 2.5%. In both genotypes the abnormal embryos displayed only initials which were not formed yet or degenerated along fruit ripeness, whereas the other quantity did not germinate or dried as soon as they germinated. NAA100 and 500, displayed 0.8% and 1.1% buds deriving not from the embryo, but from the endosperm plantula, which regenerate somatic embryos, (embryoid bodies) which formed a bud and a root (Dendrogram 1 and 2). Subsequently all the cells thanks to the genome possess enough information to create another identical tree (Thawaro et al., 2010; Guerra et al., 1998). As of data analysis NAA influenced both genotypes for their somatic development. NAA stimulated awakening and embryo germination (14.45%) more than Control. NAA, in high concentrations influenced the emergence of polyembryos and somatic embryos as an expression of the totipotence of their cells cv=63.8 dhe cv=14.4%, proved per **Prob > F,** 0.0369*. All the data showed that the regulator penetrated the embryo cells and promoted propagation and differentiation of vegetative and root meristem. Table 1, show that both genotypes had 43.66% embryonic germination from a 25.9% under regulator induction, when the stimulant was not applied. The capacity to germinate is a genetic characteristic and from this point of view both genotypes had cv with a wide amplitude (HH=60.5% - KAN=13%) cv=38.7% ***Prob > F*** *0.0369*. Finally the conclusion drawn shows that embryogenesis of olive seeds is an important physiological process which was favoured by the NAA induction in the cellular meristem.* High germination percentage resulted only when the outer endocarp structure was decomposed i.e. lowereed its resistance for embryo enlargement (Ismaili et al., 2013).

**Conclusions:**

Physiological changes of the embryo occur during the sleep period of the endocarp from harvesting to germination. During this period the endocarps totally ripen the embryo as a result of increasing the organic and hydrate acids. The phytoregulators have favourably influenced cellular activation, simultaneous regeneration of vegetative and root meristem as a phenomenon similar to both genotypes of the olea species. However genotype reaction was specific, as they were influenced by different genetic characteristics.

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