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Callogenesis and the influence of IBA on rhizogenesis of green parts of the olive

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Abstract

Macroexplants consisting of two nodes with leaves were prepared by the tip of sprigs of 20 autochthonous cvs in the olive collection of Valias. In addition to Control treatments with IBA 2g/l^{-1}, 5g/l^{-1}, and 8g/l^{-1} were applied at the beginning of spring. Morphological and physiological exchanges were controlled for the temperature of substrate and environment 24°C and 18°C and for air humidity 95%, throungh mist propagation method..The results exposed the genetic origin and endogenous predisposition as the beginner of rhizogenesis which fluctuated from 4.6% to 27%. Whereas the auxin increased rhizogenesis 28.7 - 48.9% as per concentrations compared to Control. The cultivars had good callogenesis of 43.6/ 95.4% (5>8>2>0 g/l^{-1} IBA), whereas rhizogenic capacity was average 14.7/63.6%, conditioned by hormones. Correlation between callogenesis and rhizogenesis was considerably good for cv. Kushan, Kaninjot, Freng (r^2=0.93) and average for cv Kotruvsi, u Kuq, Mixan etc (r^2=0.55). Maximum per rooting was 95.2% whereas the number of adventives roots was 8.8 At this phenophase of meristematic development use of dosage 5g/l^{-1} is more justifiable than with the two other concentrations of IBA and Control (r^2=0.94).

Keywords. Macroexplant, Rhizogenesis, Callogenesis, Olive, Morphological, Cultivar

Introduction

The physiological state of the rhizogenic potentials of the olive makes up one of the key elements for the characterization of autochthonous germplasm in the olive orchard of Valias [1, 2]. The first key issue involves identification of the endogenous capacity [11], followed by the methods of regeneration and propagation, which increase efficiency through the phytoregulators of callogenesis and hormonal acids as an exogenous factor [11]. From this point of view importance lies on the origin of the macroexplants, stages of meristematic development and the correlations with the processes of morphogenesis on callogenesis and rhizogenesis. [3, 4, 11]. This research specifically analyzes rhizogenesis of the autochthonous reserve in correlation with callogenesis and the influence that some extreme auxin concentrations have on the initial stage of cambium development.

Material and Method

The experiment was carried out during 2011-2012, for 20 genotypes of the autochthonous collection of Valias olive, with local names presented in *Tab.1*. 100 macroexplants were extracted per mother tree of each genotype, from top of the sprig, with dimensions 8-10 cm, with two- leaf-nodes in the apical part. *The research aims 4 versions:* **(i)** *Control* (ethanol + H_2O). **(ii)** IBA 2g/l^{-1}, **(iii)** IBA5g/l^{-1}, **(iv)** IBA 8g/l^{-1}. The auxin underwent the following procedure ($C_{12}H_{13}NO_2$): Active matter – meme sol. - hydroalcoholic sol 70% H_2O+ 30% ethanol. Treatment of the basic part 5 mm in the solution lasted 5 seconds. They were later installed based on a scheme « in block » in a nebulisation bank with a perlite substrate. The morpho-callo-rhizogenic processes were controlled for 70 days (F_1 and F_2).

Nebulisation was done as per « Cooling system » [8], and through autocompensative nature, 5 sec per 13-15 k/kal/cm². Temperature on the base of parts was kept 22°C (±1°C), whereas temperature of the atmosphere was 18°C. Light 12 hours with an intensity of 6000 lux.

The basic analysis consisted of: (i) degree of callogonesis in dynamics. (ii) Rhizogenesis in percentage referring to the primary material of callogenesis. (iii) Number of differentiated roots. The data were analyzed in Jmp software, for the variance (alpha=*0.05*), coefficient of variation, bivariate analysis and performance through **S**ummary statistics and diagnostics logistic result of Treatment, [7, 10].

Figure **1**. (left / right).Morphological exchange, wound closure, callus formation, root initials and rooting, during the olive cycle rhizogenesis.

Results and Discussions

Callogenesis: After cicatrisation of the wound, a thickened and hardened tissue called "callus' is formed beneath the cut. This tissue was regenerated at the cut or all over the segment of the internodal segment of the base, two weeks later until complete induction for 35 days, *Fig-1*. The callus has resulted from cellular parenchyma reproduction of the cortex and phloem, expanded internal and external to the sclerenchymatic ring. The well- formed -callus seems opalescent to ochre and of different dimensions, *Fig-1*, [2, 3, 5, 7]. The data presented in table *Tab-1*, confirm the use of IBA, which increased the size of the callus and was necessary for this process. The size of the callus was in conformity with the ranging of variants (5>8>2>0,IBA), and respectively its value in percentage; (*95.4, 94.6, 77,3 and 43,6*). It is obvious that Control had poor and slow callogenesis (43.6%). However under natural conditions there were several genotypes of a good natural callus such as; Kaninjot (55.3%), Kushan (62.4%%), Mixan (55.8%). Considering treatment with IBA proved through Tukey-cramer *lsd.1.67 alpha=0.05, Tab-1*, the genotypes; Kaninjot, KB, Kushan, Freng etc, displayed considerable size of callogenesis about 5 g/l^{-1}, and the variants were ranked as per degree of influence; (5>8>2>0 IBA). Callus tissue of the genotypes Kotruvs, Mixan, Freng, Kaninjot, Kushan, was propagated simultaneously in the presence of concentrations $8g/l^{-1}$ IBA (8>2>0IBA). Meanwhile some other genotypes as Oliv z Tir, Oliv Z.Elb, differentiated a callus of poor dimensions despite auxin application. Callogenesis increased its dimension proportionally with the concentration of auxin from $2g/l^{-1}$ up to 5 g/l^{-1} (r^2=0.96), while further increase of the concentration led to an inhibiting effect (r^2=0.84). In conclusion variability of callogenesis is related to the individual capacity of the genotypes.

Rhizogenesis: The formation of primary roots is dedicated to the quality of the olive thus parenchyma cells are modified to meristematic cells. Radicals start appearing in the cell layer of sapwood, so parnchymatic cells are modified to meristematic cells, [8, 9, 11]. Radicals have already appeared in the cell layer of sapwood next to the cambium always in the area of medullar ray, *Fig-3,* [7, 9, 11]**.** The degree of natural rhizogenesis displays considerable variation (*cv=17*), and has been influenced by the genetic features of the olive accessions. The genotypes UBT, Freng, Kushan, etc have differentiated the roots throughout the whole internodal base, thus corresponding to the primary rays where they have originated from, whereas other genotypes have differentiated the roots only in the callus of the base. Referring to the natural analysis (Control), in *Tab-1*, rooting percentage started with 4.6%, and went up to extreme levels of 27%.

Table 1. The main data of 20 olive Genotype, and the Analysis of Variance, Means and Std Deviations for the callogenesis and rhizogenesis.

Treatment	*Rhizogenesis Mean*	*Score Mean*	*Callogenesis Mean*	*N.of roots Mean*	*L.of roots Mean*	*p-Value*
2g/l-1	43.4 ±1.00 **C**	5.00	75.5±1.00 **B**	3.7±0.20 **B**	4.6±0.25 **C**	<.0001*
5g/l-1	63.6 ±1.00 **A**	11.00	86.1±1.10 **A**	5.3±0.30 **A**	5.2±0.25 **A**	<.0001*
8g/l-1	50.8±1.00 **B**	8.00	80.4±0.91 **A**	5.4±0.20 **A**	4.8±0.20 **AB**	<.0001*
Control	14.7 ±1.00 **D**	2.00	43.6±1.20 **C**	2.4±0.30 **C**	3.4±0.10 **D**	<.0001*

Levels not connected by same letter are significantly different. **Comparisons for all pairs using Tukey-Kramer HSD, q* 3.202, Alpha 0.05, Prob>F <.0001* Prob>ChiSq 0.0156***

The genotypes Ol.ZE, Ol.ZT, Pulazeqin Bregu etc, had poor rhizogeneous mass whereas Kaninjot, UBT, Kushan, Freng differentiated a considerable mass. Different from Control, IBA influenced the

hormonal endogenous equilibriums, thus favouring proportional rooting with its concentrations. Compared to Control the IBA influence on the rooting mass was 52.6%. *In table-2 and Fig-4*, IBA did not improve considerably any of the genotype rhizogenesis which had poor or zero rhizogenous mass. *(r^2=0.787)*. IBA hormonal effect on the rooting mass displayed variation from 19% to 40% more than Control, ranging as per performance of the variants: (5>8>2>0 IBA). These results are the consequence of direct IBA influence as an activator of endogenous auxins, which rebuilt new equilibriums more favourable than Control *(r^2=0.937)*, [3, 5, 11]

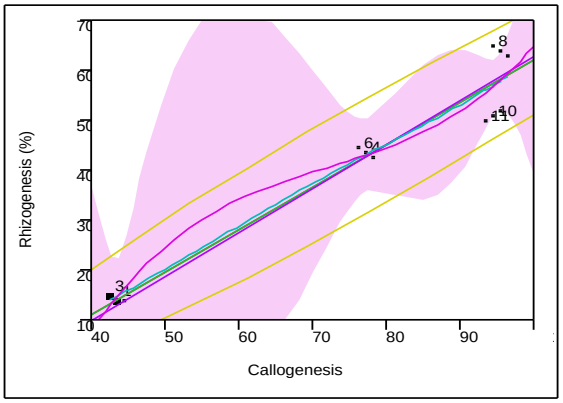

Figure 2. Bivariate Fit of Rhizogenesis (%) by Callogenesis (%) on the average of 20 olive Genotypes

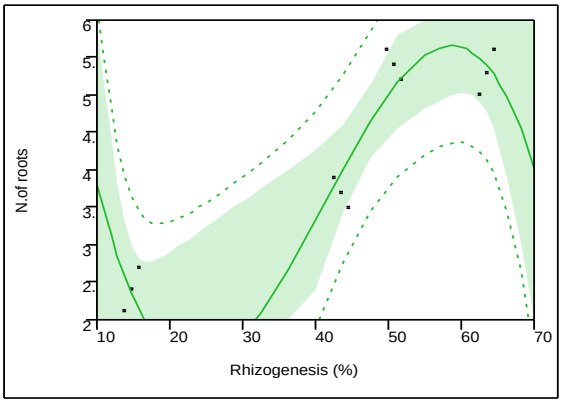

Figure 3. Rhizogenesis (%) By Number of roots, analyzed data on the average of 20 Genotype olive rhizogenesis

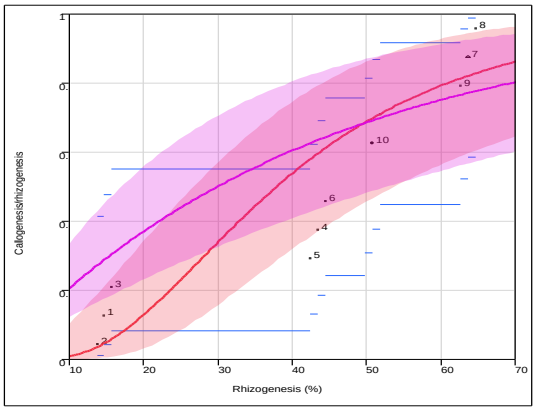

Figure 4. Analysis of coefficient of regression,Bivariate Fit of Ratio C/R by Rizhogenesis (%)

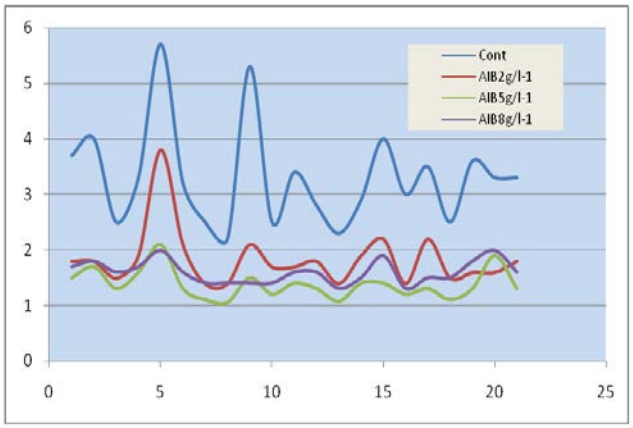

Figure 5. Bivariate fit of C/R by Treatment (%) on the average of 20 Genotype olive rhizogenesis.

Table 2. Multivariate Correlations and Parameter Estimates analyzed data on the average of 20 Genotype olive rhizogenesis.

	Callogenesis %	***Rhizogenesis %)***	***N.of roots***	***L. of roots***	***t Ratio***	***Prob>|t|***
Callogenesis %	1.0000	0.9681	0.9563	0.9454	0.06	0.9557
Rhizogenesis (%)	0.9681	1.0000	0.9189	0.9616	0.51	0.6252
N.of roots	0.9563	0.9189	1.0000	0.8995	0.30	0.7697
L. of roots	0.9454	0.9616	0.8995	1.0000	0.33	0.7518

The correlations are estimated by REML method

Rhizogenesis-callogenesis correlations – primary material. *In Figure-2, 4, 5 and Table-2*, Orthogonal analysis of regression, for the ratio Callogenesis/Rhizogenesis (C/R) displayed strong relation of positive trend, [5, 10, 11]. The ratio (C/R) became more favourable via induction of IBA 5g/l^{-1}. **Orthogonal Regression, mean,** Rhizogenesis (%) = 4.3823816 + 0.4996344*Callogenesis + 0.0088632* (Callogenesis-77.725)^2 + 0.0005442*(Callogenesis-77.725)^3, *Figure 4.* Generally different IBA concentrations caused indices of different performance as per ranging of variants (5>2>8>0IBA). Correlative indices of callogensesis and primary material resulted better with IBA5g/l^{-1} and as per ranging of variants (1.2, 1.3, 1.3, 2.3). Judging upon the rhizogenic average mass of 20 genotypes we proved that IBA5g/l^{-1} inducted rhizogenesis 63.6% , compared to the primary material. Considering this point of view IBA2 g/l^{-1} = 43.4%, IBA8 g/l^{-1} = 50.8%, whereas Control = 14.7%. Rhizogenesis of any IBA concentration was better than Control and from this viewpoint they range as follows (5>8>2>0 IBA). Rhizogenesis of each genotype had specific ratios with Callogenesis and primary material and proved to be the responsible genotype for this index. The lower the value of this ratio the better was the rhozogenic mass. With IBA5 g/l-1 concentration; Rhizogenesis - callogenesis - primary material have the normal relations 1.3 dhe 1.5, while there are reports of negative control 2.8 and 6.6.

C/R index was better for the genotypes Kaninjot, Kushan, Freng, which had a high coefficient of correlation (r^2=0.96) and average for Kotruvsi, u Kuq, Mixan cvs (r^2>0.75). In *Figura-3 and 5*, for the analysis of homogeneity and level of differentiation within the plot of authenticity displayed the performance of each hypothesis compared to Control. Considering these circumstances *IBA2* g/l^{-1}, had all its observations within the limits of authenticity with a performance of 29.5%; and respectively IBA5 g/l^{-1} = 43.2% and IBA8 g/l^{-1} had a performance of 34.5%, *Figura-4*.

Maximum per rooting was 95.4% per cv.Kushan, and 91.6%, 87% per cv Freng and Kaninjot. Whereas the number of maximal adventitious roots was 8.6 in cv. Freng. The number of roots was influenced by the olive genotype as well as the IBA concentrations. The higher number of roots corresponded with the genotypes; Freng 8.8 and Kushan 7.2 with IBA 5 g/l^{-1} and a lower Control number.

Conclusions

Mitotic cellular division caused callogenesis in the cut parts, which was considerably influenced by the IBA concentrations as well as the individual capacities of the genotype.

IBA concentrations modified different equilibriums endogenous/exogenous which controlled the stimulus of morphogenesis processes. The genotypes with the presence of IBA 5 g/l^{-1} reinforced the effect of callogenesis, caused rapid cellular propagation and differentiated voluminous callus. IBA 8 g/l^{-1}, which was dot not result really favourable for rhizogenesis, has in any case caused a considerable callogenesis mass.

Rhizogenesis has been closely related to IBA concentrations as well as to the individual characteristics of the genotype. When the index of the C/R ratio was next to the value (1) it served as a proof for good rhizogenic mass.

Application of the dosage 5g/l^{-1} at this stage of meristematic development was better justified than when compared with the two other IBA concentrations.

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