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Initial nutritional status and exogenous IBA enhanced the rooting capacity of carob (*Ceratonia siliqua* L.) cuttings under mist system.

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Abstract
Rooting is the major hurdle for carob propagation by cutting. This study investigated the effect of initial nutrient contents and exogenous indole-3-butyric acid (IBA) on the rooting capacity of herbaceous (HC) and softwood (SC) cuttings in two Moroccan populations of carob, Tighdouine and Tamellalet, under mist condition. Herbaceous and softwood cuttings of both populations were subjected to exogenous IBA 0, 5000, 6000, 7000, 8000 or 9000 mg L^{-1} during 10 s, 30 s or 60 s. Obtained results showed that without IBA treatment, cuttings were not able to make roots, while all of the treated cuttings initiated roots. Moreover, cuttings response varied depending on the IBA treatment, type of cutting and origin. The highest rooting percentage (85.2% and 65.71%) and the highest number of roots (10.33 and 9) were obtained with 7000 mg L^{-1} IBA during 60 s in SC and HC of Tighdouine population, while with 5000 mg L^{-1} in SC (9.67 roots initiated in 50.56% cuttings) and HC (6.6 roots initiated in 45.71% cuttings) of Tamellalet. Cuttings performance was positively correlated with their initial contents of total soluble sugars, P, Ca, Fe, Si, and S and negatively correlated with initial N and Na concentrations, but not correlated with initial K, Cl, and Mn levels. Thus, rooting capacity of carob cuttings is predetermined by their initial nutritional status and highly dependent on adequate treatment of exogenous IBA.

Keywords: Ceratonia siliqua L., cuttings, indole-3-butyric acid, initial nutritional status, rooting capacity

1. Introduction
Carob tree (*Ceratonia siliqua* L.) species belonging to the leguminous family has a high hardiness and adaptability to the climatic conditions of the Mediterranean basin, where it's cultivated but also wild genotypes are widespread. This tree presents interesting agro-ecological characteristics such as tolerance to drought and salinity and adaptation to soils with low inputs [1, 2]. Moreover, due to its ability to preserve and enrich soil fertility, carob cultivation facilitates the establishment of other plant species, being particularly useful for the rehabilitation of difficult areas where it can simultaneously play the role of pioneer and productive species. These characteristics together with the high economic value of its products (used in food, chemicals, cosmetics, processing etc.) [3], make this species suitable not only as a biological tool to counteract processes of erosion and desertification, but also as a challenge for the development of the marginal and sub-marginal areas in the Mediterranean basin.
However, the large-scale cultivation of carob tree is limited by the traditional methods of propagation that fail to meet the growing demand for plants with valuable characteristics. Carob tree is traditionally propagated by seeds germination or asexual propagation techniques such as grafting. Seeds are not recommended for propagation because seedlings show high heterozygosity, are slow to became reproductive and about 50% of plants are potentially nonproductive males. Moreover, due to their extremely hard coat and their difficulty to absorb water, seeds germination needs many physical and chemical scarification pretreatments [4, 5]. To date, propagation of the carob tree is conducted primarily by grafting scions of selected productive females on wild rootstocks. However, rootstocks widely vary in their growth performance and environmental adaptation. It takes

more than two years to obtain suitable grafted nursery material. Propagation by cutting is problematic because carob has been described as one of the most difficult to root species [6]. In recent years, many of such species were induced to root using exogenous auxins and culture under mist condition [7, 8]. However, this technique has not yet been fully achieved for many woody species including carob.

On the other hand, physiological condition of the mother plant such as, auxin level, rooting co-factors, carbohydrate storage, and nutrients contents exert great influence on the rooting ability of cuttings [9, 10]. Roots formation efficiency of cuttings is generally greatest when carbohydrate content of stock plants is the highest and very poor when carbohydrate storage was less [10]. In fact, soluble sugars are very important in roots formation as source of energy and carbon skeletons needed for cells regeneration [10, 11]. Moreover, plants showing excessive nitrogen content have luxuriant growth but cuttings taken from such plants exhibit poor rooting efficiency. Thus, low N and high carbohydrate balance in stock plant is necessary for better rooting. Mineral nutrients have also essential and vital functions in cells metabolism. They can function as constituents of organic structures, as activators of enzymatic reactions, or as charge carriers and osmoregulators [12]. The present investigation aims to study the effects of the initial nutritional status and the application of exogenous IBA on the rooting performance of cuttings of two Moroccan carob populations under mist system conditions.

2. Material and methods

2.1. Plant material and experimental design

The study was carried out in 2013 at Babram Nursery Society, Marrakesh (Morocco). Herbaceous shoots of 3 to 5 mm diameter of the same year and two years old 8 to 10 mm diameter softwood shoots were collected on March from ten vigorous trees of two Moroccan populations of carob 'Tighdouine' (31.28° N, 7.30° E and an altitude of 1.47 km) and 'Tamellalet' (31.47º N, 7.33° E and an altitude of 843 m). Cuttings (10 to 15 cm length with two first leaflet) bases were immersed in 0, 5000, 6000, 7000, 8000 or 9000 mg L^{-1} IBA solutions for 10 s, 30 s or 60 s. IBA solutions were prepared by dissolving IBA powder (Sigma) in ethanol (50% v/v). Each treatment consisted of three replicates with 20 cuttings in each. All cuttings were then planted in 4 cm × 4 cm × 4.5 cm alveoli filled with peat and randomly kept under intermittent mist in polyethylene greenhouse under natural light/dark conditions, 70-80% relative humidity and 30 ± 2 ºC day and 20 ± 2 °C night temperature. After two months, the rooting percentage was determined as the ratio of the number of rooted cuttings to the total number of cuttings. A sample of three cuttings per replicate was also taken to determine the number of roots per cutting. Rooted cuttings were then transplanted into plastic bags (15 cm × 20 cm) containing 1.5 kg of a mixture of sand and soil (2:1) and transferred to hardening greenhouse.

2.2. Initial mineral nutrients and soluble sugars contents

At the cuttings collecting time, leaf samples were taken from cuttings of each population and stored at -20 ºC for initial nutrients and total soluble sugars (TSS) determination.

For the total soluble sugars measurement, fresh leaves of 100 mg were grounded with a mortar and pestle in 50 mM potassium phosphate buffer (PBS). The homogenate was filtered using filter paper and centrifuged at 38,720×g for 10 min at 4 °C. A 0.1 mL aliquot of PBS extract was added to 3 mL of freshly prepared anthrone reagent (200 mg anthrone, 100 mL 72% H_2SO_4) and the mixture was heated in boiling water bath for 10 min [13]. After cooling, the absorbance was measured at 620 nm. TSS content was calculated using glucose standard curve.

To determine mineral nutrient contents, fresh leaves were oven dried at 80 °C for 48 h and then grounded in a mortar. The concentrations of Fe, Cl, Si, S and Mn were determined by X-Ray diffraction using a portable XRF analyzer (Olympus NDT, Waltham, USA). For P, K, Na and Ca analysis, dry matter was incinerated at 500 °C for 5 h and then digested in 2 M HCl. P content was determined using the molybdate blue method according to Murphy *et al* (1962) [14], while K, Na and Ca concentrations were measured using flame spectrophotometer (model AFP100, Biotech Engineering Management Co. Ltd., UK) according to Brown *et al*. (1946) [15]. N content was determined using Kjeldhal method [16].

2.3. Statistical analysis

Data were statistically analyzed with IBM SPSS 20.0 software using the analysis of variance to several factors. Initial nutrient contents, cutting type, concentration of IBA and time of IBA application were respectively used as first, second, third and fourth factors. Significance differences and interaction between factors were calculated at 5 %. Comparison of means values was carried out using Newman-Keuls (SNK) test. The results were expressed as means ± standard errors, and $P \leq 0.05$ was considered statistically significant.

3. Results and discussion

As is the case with other plants species, the multiplication of carob tree by cutting consists in separating cuttings from the mother plant and culture them in conditions such it could make roots and regenerate entire plantlets. In last decades, substantial progress has been made in the rooting of difficult hardwoods such as eucalyptus [17] and date palm [7, 8] using exogenous auxins and mist system. Currently, indole-3-butyric acid (IBA) is the most widely used auxin to stimulate rooting process in cuttings because of its high ability to promote root initiation [18] and its weak toxicity and great stability in comparison to 1-naphthaleneacetic and indole-3-acetic acid [19]. In the present study, analysis of variance revealed that rooting capacity of carob cuttings varied significantly depending on cuttings initial nutrient contents, cuttings type, IBA treatment (time and concentration) and interaction between these factors (Table 1).

Table 1: Analysis of variance of the effect of initial nutrient contents, cuttings type, concentration and time of exogenous IBA application on the rooting percentage and the number of roots per cutting of two Moroccan carob populations 'Tighdouine' and 'Tamellalet'.

Source of variance	Number of roots per cutting	Rooting percentage
Initial nutrient contents (A)	12.00 **	805.483 ***
Cutting type (B)	67.69 ***	93.947 ***
Concentration of IBA (C)	261.35 ***	721.692 ***
Time of IBA application (D)	94.32 ***	553.000 ***
A × B	0.33 NS	12.515 ***
A × C	50.09 ***	364.911 ***
A × D	3.48 *	38.616 ***
B × C	59.65 ***	48.909 ***
B × D	5.92 **	157.265 ***
C × D	13.80 ***	86.829 ***
A × B × C	17.34 ***	18.453 ***
A × B × D	6.10 **	64.514 ***
A × C × D	12.49 ***	86.962 ***
B × C × D	9.23 ***	46.180 ***
A × B × C × D	12.07 ***	73.168 ***

NS: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

This study revealed that rooting capacity of carob cuttings was very sensitive to exogenous IBA application, while 85.2% of rooting was induced by 7000 mg L^{-1} IBA (the highest percentage among the treatments), no root induction was recorded in untreated cuttings (Table 2). These data showed that exogenous IBA is substantially required to induce adventitious roots formation in carob cuttings as it was observed in other plant species [7, 8, 20-23]. Jackson (1986) [24] reported that the rooting ability of cuttings is largely determined by the balance between endogenous promoters and inhibitors of rhizogenesis. In this experiment, untreated cuttings developed stems and leaves but not roots. This indicates that without IBA, the endogenous hormonal balance was probably more suitable for the development of shoots. Thus, exogenous IBA may help in changing the endogenous level of growth regulators to establish an appropriate hormone balance for root proliferation. This is evidenced by the high rooting performance of cuttings pretreated with IBA. In fact, it well established that auxins play a key role

in plant propagation since it regulates cells differentiation and are the most known promoters of roots formation [21].

Table 2: Influence of concentration and time of exogenous IBA application on rooting percentage of softwood (SC) and herbaceous (HC) cuttings of two Moroccan carob populations 'Tighdouine' and 'Tamellalet'.

IBA (mg L^{-1})	Time (s)	Rooting percentage (%)			
		Tighdouine (SC)	Tighdouine (HC)	Tamellalet (SC)	Tamellalet (HC)
Non-IBA		0.00 z	0.00 z	0.00 z	0.00 z
5000	10	27.78 ± 0.62 i-q	15.23 ± 0.55 luv	7.41 ± 0.00 wxyz	21.06 ± 1.10 p-t
	30	33.70 ± 0.00 jkl	22.86 ± 0.55 o-s	50.00 ± 1.23 e	33.33 ± 0.55 jkl
	60	40.74 ± 1.23 ghi	23.80 ± 0.55 o-s	50.56 ± 1.23 d	45.71 ± 0.00 efg
6000	10	22.22 ± 0.00 pqrs	26.66 ± 1.10 m-r	1.85 ± 0.00 yz	13.33 ± 0.55 vw
	30	59.26 ± 1.23 cd	42.86 ± 1.45 fgh	16.67 ± 0.36 stuv	34.29 ± 0.95 ijkl
	60	42.59 ± 1.85 fgh	40.00 ± 0.95 ghi	27.78 ± 1.23 i-q	34.29 ± 1.85 ijkl
7000	10	22.22 ± 0.36 pqrs	47.61 ± 1.10 ef	11.11 ± 0.00 vwx	22.86 ± 0.95 o-s
	30	22.22 ± 1.23 pqrs	54.29 ± 0.95 d	11.11 ± 1.23 vwx	11.43 ± 0.00 vwx
	60	85.19 ± 1.85 a	65.71 b ± 1.90	11.11 ± 0.62 vwx	22.86 ± 0.95 o-s
8000	10	5.56 ± 0.62 xyz	34.29 ± 1.23 ijkl	7.41 ± 0.36 wxyz	20.00 ± 1.90 rstu
	30	7.41 ± 0.00 wz	22.86 ± 0.00 o-s	12.96 ± 0.36 vw	14.29 ± 0.95 uvw
	60	61.11 ± 2.17 bc	11.43 ± 0.55 vwx	37.04 ± 0.95 hij	28.57 ± 0.95 k-p
9000	10	7.41 ± 1.23 wxyz	29.52 ± 1.45 k-o	16.67 ± 0.62 stuv	14.29 ± 0.00 uvw
	30	35.19 ± 1.23 ijk	32.38 ± 0.55 jklm	0.00 z	25.71 ± 1.45 m-r
	60	31.48 ± 0.00 j-n	45.71 ± 1.90 efg	0.00 z	11.43 ± 0.55 vwx

Mean values ± SE followed by the same lower-case letters are not significantly different at $P \leq 0.05$ by Newman-Keuls test.

However, it is always challenging to adjust the appropriate hormonal balance to successfully induce adventitious root. Several explanations were suggested to clear the promoting effects of exogenous auxin on roots formation. Auxin may i) enhance cell division and differentiation in the vascular cambium, leading to the formation of roots [21], ii) antagonize the effects of other hormones which can inhibit rooting such as gibberellins and cytokinins [6], or iii) stimulate redistribution and mobilization of some auxin cofactors and carbohydrates towards the base of cuttings [1, 25]. Our data have also shown that cuttings of the two populations of carob significantly ($p < 0.001$) varied in their response to IBA concentrations. Cuttings of Tighdouine showed higher rooting ability than those of Tamellalet regardless of IBA treatment. The highest rooting percentages (85.2% and 65.7%) were obtained with 7000 mg L^{-1} IBA during 60 s respectively in softwood (SC) and herbaceous (HC) cuttings of Tighdouine. Whereas, for Tamellalet, the highest rooting percentages (55.6% for SC and 45.7% for HC) were obtained with 5000 mg L^{-1} IBA during 60 s (Table 2). Furthermore, these IBA treatments significantly ($p < 0.001$) presented more roots per rooted cutting respectively in Tighdouine (10.33 roots per SC and 9 roots per HC) and Tamellalet (10.67 roots per SC and 6.67 roots per HC) (Table 3).

It seems that the appropriate hormonal balance was found to be achieved with 7000 mg L^{-1} IBA for cuttings of Tighdouine and with 5000 mg L^{-1} IBA for those of Tamellalet. This difference in response to IBA application between the two populations may be related to differences in tissue sensitivity to auxin and / or in the level of endogenous growth regulators [11, 21]. Differences in rooting capacity between the two carob populations may depend also on other physiological and/or biochemical characteristics of the mother plants. Root initiation involves division and differentiation of specific cells in the vascular cambium leading to the formation of roots. In such a case, physiological activities including energy reserves and nutrients status may necessarily be involved in these metabolic processes.

Table 3: Influence of concentration and time of exogenous IBA application on the number of roots per softwood (SC) and herbaceous (HC) cutting of two Moroccan carob populations 'Tighdouine' and 'Tamellalet'.

IBA (mg L^{-1})	Time (s)	Number of roots per cutting			
		Tighdouine (SC)	Tighdouine (HC)	Tamellalet (SC)	Tamellalet (HC)
Non-IBA		0.00 r	0.00 r	0.00 r	0.00 r
5000	10	2.67 ± 0.19 opqr	3.33 ± 0.19 m-q	3.00 ± 0.33 nopq	4.00 ± 0.33 l-q
	30	2.33 ± 0.19 pqr	4.67 ± 0.19 j-p	8.67 ± 0.51 b-f	5.67 ± 0.38 g-n
	60	7.00 ± 0.00 d-k	4.33 ± 0.19 k-q	9.67 ± 0.51 bcd	6.67 ± 0.19 e-l
6000	10	8.00 ± 0.00 b-h	5.33 ± 0.19 h-o	1.67 ± 0.19 qr	1.67 ± 0.19 qr
	30	7.33 ± 0.51 d-j	6.00 ± 0.00 f-m	7.00 ± 0.33 d-k	5.00 ± 0.33 i-p
	60	8.33 ± 0.51 b-g	5.67 ± 0.38 g-n	9.00 ± 0.33 bcde	6.00 ± 0.00 f-m
7000	10	8.67 ± 0.53 b-f	4.00 ± 0.33 l-q	8.33 ± 0.38 b-g	2.67 ± 0.38 opqr
	30	10.67 ± 0.51 ab	4.33 ± 0.19 k-q	5.67 ± 0.38 g-n	3.33 ± 0.51 m-q
	60	10.33 ± 0.38 abc	9.00 ± 0.33 bcde	9.67 ± 0.38 bcd	4.67 ± 0.38 j-p
8000	10	2.33 ± 0.38 pqr	4.33 ± 0.19 k-q	8.67 ± 0.19 b-f	3.00 ± 0.33 nopq
	30	6.33 ± 0.38 e-l	4.00 ± 0.00 l-q	5.67 ± 0.51 g-n	6.33 ± 0.19 e-l
	60	8.00 ± 0.33 b-h	5.67 ± 0.38 g-n	6.67 ± 0.51 e-l	7.33± 0.51 d-j
9000	10	7.67 ± 0.51 c-i	7.33 ± 0.38 d-j	5.67 g-n	6.00 ± 0.33 f-m
	30	8.00 ± 0.00 b-h	9.00 ± 0.57 bcde	0.00 r	8.00 ± 0.00 b-h
	60	8.00 ± 0.33 b-h	8.33 ± 0.38 b-g	0.00 r	8.33 ± 0.38 b-g

Mean values ± SE followed by the same lower-case letters are not significantly different at P ≤0.05 by Newman-Keuls test.

In the current study, rooting capacity of carob cuttings significantly varied according to their initial nutrient contents (Table 4). Initial TSS, P, Ca, Fe, Si and S concentrations were higher in cuttings of Tighdouine than in those of Tamellalet, while N and Na concentrations were higher in cuttings of Tamellalet population. However, K, Cl and Mn contents didn't change significantly between the two populations.

Table 4: Initial total soluble sugars (TSS) and mineral nutrients contents of cuttings of two Moroccan carob populations 'Tighdouine' and 'Tamellalet'.

		Cuttings of Tighdouine	**Cuttings of Tamellalet**	**Analysis of variance**
TSS (mg g^{-1}FW)		61.43 ± 0.89	55.55 ± 0.52	10.85 *
Nutrients contents (mg g^{-1} DW)	N	7.17 ± 0.07	13.40 ± 0.14	517.97***
	P	0.71 ± 0.00	0.49 ± 0.00	414.45***
	K	3.90 ± 0.08	3.50 ± 0.05	5.58 NS
	Ca	7.97 ± 0.18	5.92 ± 0.17	22.92 **
	Na	3.39 ± 0.04	12.69 ± 0.04	8765.87**
	Cl	27.30 ± 0.10	27.00 ± 0.10	5.79 NS
	Fe	0.25 ± 0.00	0.19 ± 0.00	972.35***
	Si	0.56 ± 0.02	0.50 ± 0.02	184.32***
	S	0.21 ± 0.02	0.18 ± 0.00	83.12**
	Mn	0.05 ± 0.00	0.05 ± 0.00	0.08 NS

NS: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

The high rooting ability exhibited by cuttings of Tighdouine was positively correlated with their initial levels of TSS, P, Ca, Fe, Si and S and negatively correlated with their initial levels of N and Na (Table 5).

Table 5: Pearson correlations between rooting percentage of cuttings and their initial nutrient contents

Pearson correlation	
	Rooting percentage
TSS	0.840*
N	-0.967**
P	0.949**
K	0.703 NS
Ca	0.911*
Na	-0.969**
Cl	0.755 NS
Fe	0.979**
Si	0.964**
S	0.950**
Mn	-0.145 NS

NS: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

The importance of initial soluble sugar and some particular nutrients on rooting ability has been investigated by many researchers [9, 10, 26]. Denaxa *et al*. (2012) [10] reported that the highest rooting percentage achieved by Arbequina olive cultivar was directly related to its high soluble sugars content. Tsipouridis *et al*. (2003) [9] suggested that high initial Fe content induced high rooting percentage in peach cuttings, while Dag *et al*. (2012) [26] reported that with high level of P olive cuttings presented great rooting ability. However, both investigations showed that high N content coincides with low rooting capacity. Soluble sugars can promote root initiation and development by diverse mechanisms, i) provide the energy and carbon skeletons needed for root regeneration [27, 28], ii) strongly interact with plant hormone signaling [29], iii) modulate gene activity [30] and iiii) provide co-delivery of other components required for adventitious root formation [31]. Moreover, P, Ca and Fe might have promotive effect on root regeneration due to their involvement in many physiological processes leading to new cells formation. Phosphorus is a constituent of many essential components (nucleic acids, phospholipids, phosphoproteins, dinucleotides and adenosine triphosphate) and required for other processes including energy transfer and regulation of many enzymes. Calcium plays a key role in cell division, auxin transport and cell-wall synthesis and stability [19, 32]. By acting as a co-factor for many enzymes, involved in fundamental processes such as DNA and hormones synthesis, Fe plays a key role in the induction of roots formation.

In the contrary, in this study the low rooting ability of cuttings coincide with high initial concentrations of N and Na. This negative correlation could be explained by the fact that high levels of N can induce abundant growth which results in reduced soluble carbohydrate reserves or other components and cofactors essential for rooting [6]. Na toxicity in cells can influence or inhibit some physiological processes necessary for cells formation. However, the exact reason why high contents of N and Na result in poor rooting remains unknown.

Conclusions

In this study, carob, a difficult to root species, was successfully propagated by cuttings taken from mother plants exhibiting a good physiological and nutritional status and using suitable IBA treatment. It highlighted the essentiality of adequate exogenous IBA treatment and the importance of the initial soluble sugars and mineral

nutrients contents for the induction of adventitious roots formation in carob cuttings. High rooting percentage (85.2% and 65.71%) and high number of roots (10.33 and 9) were obtained using treatment with 7000 mg L^{-1} IBA during 60 s in SC and HC of Tighdouine which showed high initial contents of total soluble sugars, P, Ca, Fe, Si, and S, but low initial N and Na concentrations. Thus, rooting capacity of carob cuttings is predetermined by their initial nutritional status and highly dependent on adequate treatment of exogenous IBA.

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References

1. Sakcali M. S., Ozturk M., *J. Arid. Environ*. 57(2) (2004) 141.
2. Ozturk M., Dogan Y., Sakcali M.S., Doulis A., Karam F., *J. Environ. Biol.*, 31 (2010) 233.
3. Fadel F., Fattouch S., Tahrouch S., Lahmar R., Benddou A., Hatimi, A., *J. Mater. Environ. Sci.* 2(3) (2011) 285.
4. Esma G., Hamide G., Dilek Y., *Bull. UASVM. Hort*. 66(1) (2009) 687.
5. Pérez-garcía F., *Span. J. Agric. Res.* 7(2) (2009) 398.
6. Hartmann H. T., Kester D. E., Davies F.T., Geneve R. L., New Jersey, U.S.A. (1997) 239.
7. Qaddoury A., Amssa M., *Acta. Bot. Gallica.* 150(2) (2003) 213.
8. Qaddoury A., Amssa M. *Bot. Bull. Acad. Sin*. 45 (2004) 127.
9. Tsipouridis C. G., Thomidis T. *Hor.t Sci. (prague).* 30 (2003) 108.
10. Denaxa N. K., Vemmos S. N., Roussos P. A., *Sci. Hortic*. 143 (2012) 19.
11. Moshtaghi E.A., Shahsavar A. R., *J. Biol. Environ. Sci*. 4 (12) (2010) 143.
12. Marschner H. Academic Press, London. (1995).
13. Irigoyen J. J., Emerich D. W., Sanchez-diaz M., *Physiol. Plantarum*. 84(1) (1992) 55.
14. Murphy J., Riley J. P., *Anal. Chim. Acta*. 27 (1962) 31.
15. Brown J.G., Lilleland O., *Proc. Amer. Soc. Hort. Sci.* Alexandria. (1946) 341.
16. Nelson D. W., Sommers L. E., *Agron. J*. 65(1) (1973) 109.
17. Marques C. M., Vasquez-kool J., Carocha V. J., Ferreira J. G., Omalley D. M., Liu B. H., Sederoff R., *Theor. Appl. Genet*. 99(6) (1999) 936.
18. Weisman Z., Riov J., Epstein E., *Plant. Physiol*. 91(3) (1988) 1080.
19. Blazich F. A., In the Davis T. D., Haissig B. E., Sankhla., N., eds. Dioscorides Press. Portland, Oregon (1988).
20. Mousa A. A. K., *Pak. J. Biol. Sci.* 6(24) (2003) 2040.
21. Krisantini S., Johnston M., Williams R. R., Beveridge C., *Sci. Hortic.* 107(2) (2006) 171.
22. Cristofori V., Rouphae Y., Rugini E., *Sci. Hortic*. 124(2) (2010) 189.
23. Contessa C., Valentini N., Botta R., *Sci. Hortic*. 131 (2011) 103.
24. Jackson M.B., Martinus. Nijhoff. Publ. Dordrecht (1986).
25. Husen A., Pal M., *New. Forest*. 33(3) (2007) 309.
26. Dag A., Erel R., Ben-gal A., Zipori I., Yermiyahu U., *Hor. Sci*., 47(2) (2012) 307.
27. Li M., Leung D. W. M., *Plant. Growth. Regul*. 19(4) (2000) 423.
28. Calamar A., De klerk G. J., *Plant. Cell. Tiss. Org*. 70(2) (2002) 207.
29. Leon P., Sheen J., *Trends. Plant. Sci*. 8(3) (2003) 110.
30. Koch K.E., *Annual. Review. Plant. Biol*. 47(1) (1996) 509.
31. Druege U., Zerche S., Kadner R., Ernst M., *Ann. Bot-London*. 85(5) (2000) 687.
32. Bellamine J., Penel C., Greppin H., Gaspar T., *Plant. Growth. Regul.* 26 (1998) 191.