

Genotypic and Growth Regulator Combination Effects on *in vitro* Nodal Segments of Jojoba (*Simmondsia chinensis* (Link)

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Abstract: The genotypic and BAP and kin in combination with NAA, IAA and 2,4- D effects on *in vitro* nodal segments of jojoba were investigated. The 1.0 - 2.0 cm nodal segments were prepared from the newly established lateral branches on the 5 years old jojoba plants. Shoot initiation and elongation was obtained using the MS Basel medium supplemented with 0.5g/l⁻¹ activated charcoal. The genotype "Hada Al-Sham" produced the highest no. of regenerated shoots, no. of nodes/shoot, length of primary shoots and no. of shoots/explants. Jojoba genotypes 'Medina' and 'Hael B' recorded the least values of shoot formation and regeneration at all tested growth regulator combination. The culture medium supplemented with high BAP and IAA concentration enhanced shoot formation of the nodal segments of the four tested jojoba genotypes. The maximum length of regenerated shoots, no. of nodes/shoot and no. of shoots /explants were obtained on the culture medium MS + 10 µM BAP + 5 µM IAA. Callus formation observed when the nodal segments were cultured on MS + 10 µM BAP + 10 µM NAA and MS + 10 µM BAP + 10 µM IAA. Nodal segments of the tested genotypes were not stimulated on culture medium supplemented with BAP in combinations with 2, 4-D and kin alone and its combination with NAA, IAA and 2,4-D.

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Abbreviations: BAP: Benzyl Amino Burien; NAA: Naphthalene Acetic Acid; 2,4- D: ,24- Dichlorophenoxyacetic; MS: Murashige and Skoog; IAA: Indole acetic acid; Kin: Kinten µM: MicroMole; PGRs: Plant growth regulators; B5: Gamborg B5 Medium; BA: Benzyl adenine; IBA: Indole butric acid; GA3: Gibberellic acid; Activated Charcoal (AC); CR: Completely Randomized design

1. Introduction

Jojoba (*Simmondsia chinensis*) was introduced to cultivation in various semi-arid and arid regions of the world due to its low water requirements, root system and economical potential. Jojoba was introduced first time in Saudi Arabia in 1993(Osman and Abo Hassan, 1997), however there are few information about the cultivated area and productivity (FAOSTATE, 2010). The seeds of jojoba contain about 50–55% distinctive which is oil stable under high pressure and temperature, un-saturated and simple on the molecular base. These characteristics are amenable to be used commercially in the cosmetic, pharmaceutical and lubricant industries (Gentry, 1958) . Sexual propagation of jojoba dose not reproduce true to type plants and asexual propagation of single jojoba plant is necessary to conserve the desirable traits. Several asexual propagation techniques were used to reproduce jojoba, e.g. layering (Reddy, 2003), grafting (Bashir *et al.*, 2007), and cuttings (Singh *et al.*, 2003). However, the availability of a maximum number of possible propagules and seasonal propagation are the major

challenges of these techniques. Micropropagation is a tissue culture technique suited to the multiplication of elite varieties by using plant leaf, root, and stem explants. The major advantageous of this technique are the production of pathogen-free plants, that can be used for genetic improvement of the species and provide a commercial production within a restricted time and space (Agrawal *et al.*, 2002). There are few studies considered the genotypic effects and growth regulators combination on *in vitro* propagation of jojoba using nodal segments explants. Generally, MS and B5 media supplemented with a combination of cytokinins (BA, Kinetin and Zeatin) and auxins (NAA, IAA, IBA) have been used (Agrawal *et al.*, 2002; Bashir *et al.*, 2007). MS media supplemented with BA and GA3 partially enhanced shoot initiation of jojoba shoot tips, while MS + IBA supported root formation (Heba-Allah, 2009). Bud initiation and shoot multiplication were found to be greatest on Murashige and Skoog's (MS) medium supplemented with BA and adenine (Singh *et al.*, 2008). Encapsulated buds exhibited the best shoot development on Murashige and Skoog (MS) medium supplemented with 1.0 mg/l

BA, 40 mg/l adenine sulfate and 3.0 mg/l IAA and gelled with 0.8% bacteriological agar (73% +5.17 conversion) (Hassan, 2003). The present study aimed to consider the genotypic effects and growth regulator combination on *in vitro* nodal segments of four jojoba genotypes adapted to western regions of Saudi Arabia.

2. Material and Methods

The response of four jojoba genotypes called 'Hail A', 'Madinah', 'Hail B' and 'Hada Al-sham' to *in vitro* culture using nodal segment explants was investigated Osman and Abo Hassan, (1997). The 1 – 2 cm nodal segments were prepared from the newly established branches (one year old) on the 5 years old jojoba plants. The selected branches were divided to small pieces each of 2 to 3 nodes after removing the leaves and keep the petiole intact. The branches pieces were subjected to a sterilization protocol as following: a) the explants were thoroughly washed in running tap water for 30 min, b) after dividing the branches pieces to 1 – 2.0 cm long nodal segments (each contained one node), the explants were dipped in 98% ethanol (v/v) for 5 – 10 s, c) then dipped in 10% Clorox (v/v) solution + 2 drops of Tween-20 for 15 min, and e) the explants were dipped in 0.1% mercuric chloride (w/v) solution for 15 min and subsequently washed 4 times in sterile distilled water each for 5 min. The sterilization steps from b to e were conducted in a laminar air flow hood. The explants were cultured on MS medium (Murashige and. Skoog 1962) containing 30 g/l sucrose and 8 g/l agar. The pH of the medium was adjusted to 5.8 prior to adding agar and the medium was autoclaved at 121°C and 1.05 kg cm⁻² for 15 min. The growth regulator was added to the autoclaved media through membrane filters (Millex-GS 0.22 µm filter unit) under sterilized conditions. Three concentrations of BAP and kin 1µM, 3µM and 5µM and their combination with 5µM and 10µM of NAA, IAA and 2,4- D were applied (18 treatments). The culture medium were divided into 12 ml per culture tube, then the tubes were plugged with a thermo lid and the lids were bundled with one layer of parafilm (Pechiney Plastic Packaging, Chicago, IL. 60631, USA). The nodal segments were cultured on the culture medium under laminar air flow hood. 54 explants were cultured from each genotype per replicate (3 explants/treatment x 18 treatments x 3 replicates = 162 explants/genotype x 4 genotypes = 642 explants over the experiment). The cultured tubes (total of 642 tubes) were incubated under 16 h light (white fluorescent light with intensity of 55 µmol m⁻²s⁻¹) and 8 h dark, and 24 ± 1°C temperature. The experiment was conducted in a 2 factors Completely Randomized design (CR) using 3 replications. A sub-culturing at 6 weeks interval was applied onto fresh medium of the same composition to preserve the

cultures. The following parameters were assessed: a) no. of days to bud initiation, no. of shoots per explants, no. of nodes per shoot and length of primary shoot (cm). Analysis of variance related to CR experiments was conducted as described by (Gomez and Gomez, 1984). The treatment means were compared by employing Duncan's Multiple Range test at 5% probability.

3. Results and Discussion

There were observed significant differences due to the genotypic and growth regulator effects and their interaction on *in vitro* nodal segments of jojoba at all assessed parameters (Table 1). Nodal segment explants of the used jojoba genotypes showed no responses on culture medium supplemented with kin, kin in combinations with NAA, IAA and 2,4-D, and BAP in combinations with 2, 4-D. The number of days required for bud initiation was significantly reduced when the nodal segment explants of the jojoba genotype 'Hada Al-Sham' were cultured on medium supplemented with 10 µM BAP alone (24.47 day) and 10 µM BAP + 10 µM IAA (25.43 day) (Fig 1A). Bud initiation of the jojoba genotypes 'Hail A', 'Madinah' and 'Hail B' explants was significantly postponed on culture medium supplemented with 1 µM BAP + 5 µM NAA and 5 µM BAP + 5 µM NAA, and medium without growth regulator (MS0). Moreover, the genotype 'Hada Al-Sham' recorded the maximum number of days for bud initiation with culture medium MS + 1 µM BAP + 5 µM NAA and MS + 5 µM BAP + 5 µM NAA (Fig 1A). The types and concentration of cytokinins/auxins, culture media, explants and genotypes were the most important factors affecting the number of days to bud sprouting (Bashir *et al.*, 2007; Heba-Allah, 2009). Our results showed that the BAP and its combinations with NAA decreased the number of days required to bud sprouting of jojoba genotypes which were partially in agreement with that observed by (Elhag *et al.*, 1998). The authors recorded a significant genotypic, growth regulator and medium type effects on shoot-tip culture of jojoba. They also observed that higher concentrations of BAP, NAA and IAA combinations were enhanced bud sprouting, which provided adequate time to grow the shoot and achieve maximum length than lower concentrations. 1µM BAP + 5µM IAA Length of primary shoots (cm) was significantly differed due to jojoba genotypes and applied growth regulator combination (Table 1). The highest lengths of primary shoots were 7.3cm, 6.80 cm and 6.6 cm and produced by genotype 'Hada Al-Sham' on culture medium supplemented with 5 µM BAP + 10 µM IAA, 5 µM BAP + 10 µM NAA and 5 µM BAP + 5 µM IAA, respectively. BAP alone at 1µM and 5µM reduced significantly the length of

primary shoots of the nodal segment of genotypes 'Hail B' and 'Al-Madinah'. The least values of length of primary shoots were 1.27cm and 1.36cm and produced by nodal segments of genotype 'Hada Al-Sham' when cultured on medium supplemented with 1 μ M and 5 μ M BAP, respectively (Fig 1B). Singh and Singh, (2005) and Benzioni *et al.* (2003) reported that shoot length was affected by cytokines/auxins ration, type of vessels, cultural conditions and plant genotype. The obtained results were partially in agreement with the findings of Scaramuzzi and D'Ambrosio (1988). They observed that the higher concentration of BA and NAA combinations (4.4 μ M BA + 5.4 μ M NAA) suppressed rooting but increased shoot length of plantlets. However, the results of the present study were in contrary with that reported by (Bashir *et al.*, 2007) and (Elhag *et al.*, 1998). The authors observed that BAP + auxins in lower concentrations were increased shoot length than the higher concentrations. The number of nodes per shoot was significantly increased when nodal segments of the jojoba genotype 'Hada Al-Sham' were cultured on medium supplemented with 5 μ M BAP + 10 μ M IAA (5.57 nodes/shoot). Also, explants of the genotype 'Hada Al-Sham' produced high number of nodes per shoot with culture medium 10 μ M BAP + 5 μ M IAA (5.20 nodes/shoot), 5 μ M BAP + 5 μ M IAA (5.00 nodes/shoot) and 5 μ M BAP + 10 μ M NAA (5 nodes/shoot). The culture medium without growth regulator and medium with low BAP concentration significant reduced number of nodes per shoot of explants from the jojoba genotypes 'Al-Medinah' and 'Hail B'. The minimum number of nodes per shoot was 1.23, 1.30 and 1.33 and produced by explants of the genotype 'Hail B' with culture medium without growth regulator (MS0) and 'Hada Al-Sham' with culture medium 1 μ M BAP + 10 μ M NAA and MS0, respectively (Fig 1C). The results of the present study indicated that the higher concentrations of BAP in

combination with NAA and IAA significantly increased number of nodes/shoot. There were published few studies of the effects of genotypic and growth regulator on number of nodes/shoot of jojoba. However, (Agrawal *et al.*, 2002) reported that a very few number of nodes/shoot from female jojoba clone (EC 33198) when cultured on MS medium supplemented with BA at 20 μ M. Moreover Khanam *et al.* (1999) observed that BA + IAA were better than BA+ NAA in increasing number of nodes/shoot and shoot length which was partially in agreement with the results of the present study. Regarding number of shoots per explant, nodal segments of the jojoba genotype 'Hada Al-Sham' produced the highest number of shoots per explant (8.53) on culture medium 5 μ M BAP + 10 μ M IAA. The number of shoots per explant significantly reduced when nodal segments of the jojoba genotypes 'Al-Medinah' and 'Hail B' were cultured on MS0 (without growth regulator), MS + 1 μ M BAP and MS + 5 μ M BAP. The least number of nodes per shoot was 1.16, 1.20 and 1.27 and produced by explants of jojoba genotype 'Hail B' on culture medium MS0 (without growth regulator), MS + 1 μ M BAP and MS + 5 μ M BAP, respectively (Fig 1D). The higher concentration of BAP + IAA and partially BAP + NAA produced the highest number of shoots/explants. These results may attribute to the early bud sprouting, highest shoot length (cm) and number of nodes/shoots that were produced on culture medium supplemented with high concentrations of BAP + IAA and BAP + NAA. Khanam *et al.* (1999) and (Bashir *et al.*, 2007) found that the higher concentration of BA in combinations with lower concentrations auxins was better in increasing number of shoots than those in higher ones. The results of the presented study are partially in lines with the findings of (Elhag *et al.*, 1998), who reported that higher BA/IAA ratio increased shoot multiplication.

Table-1. Mean squares for the genotypic and growth regulator effects on number days to bud sprouting, length of primary shoots (cm), number of nodes per shoots, and number of shoots per explants of the *in vitro* nodal segments of jojoba

Source of variance	df	No. of days to bud sprouting	Length of primary shoot(cm)	No. of nodes/shoots	No. of shoots /explants
Genotype (G)	3	146.85**	91.15*	32.71***	83.70*
Growth regulator (RG)	15	98.25*	5.74***	6.36*	8.37**
G x RG	45	16.44**	1.05*	0.58***	1.44**
Error	128	1.85	0.04	0.30	0.07

* Significant at $P \leq 0.05$, ANOVA, ** Significant at $P \leq 0.01$, ANOVA, ***Significant at $P \leq 0.001$, ANOVA

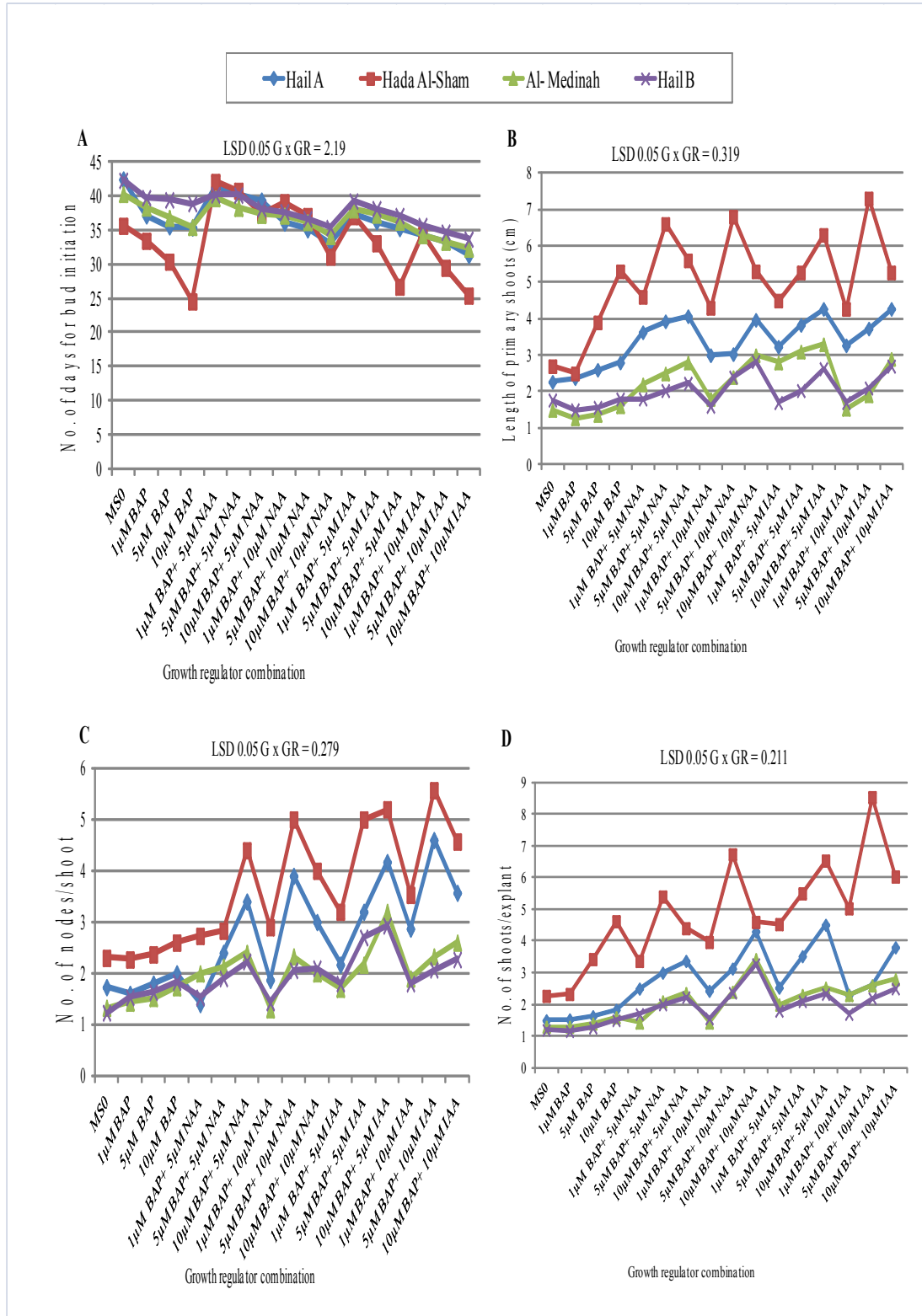


Fig-1: Genotypic and growth regulator effects on *in vitro* nodal segments of jojoba: A) number days to bud sprouting, B) length of primary shoots (cm), C) number of nodes per shoots, and D) number of shoots per explants.

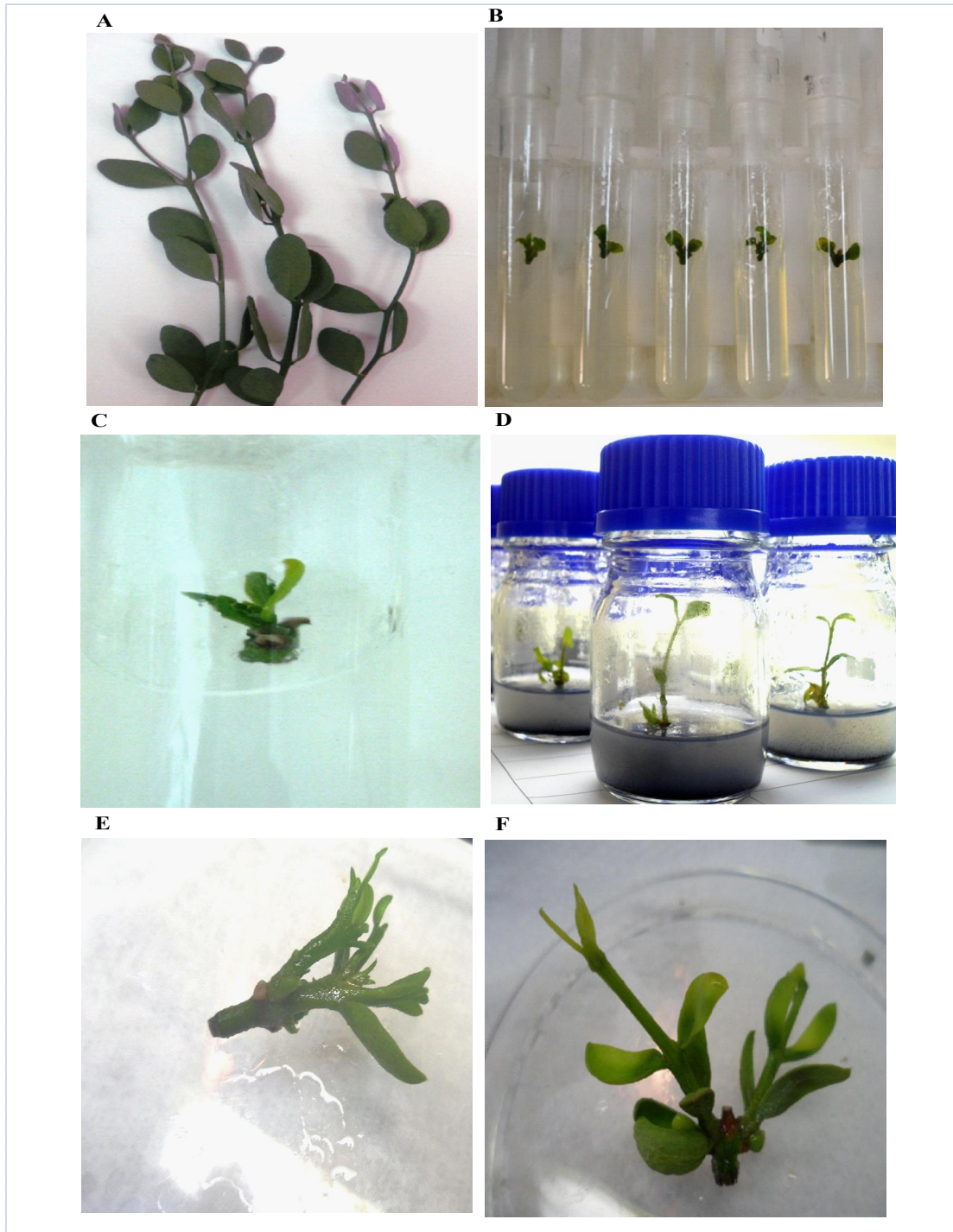


Fig-2: Genotypic and growth regulator effects on *in vitro* nodal segments of jojoba: A) the established one year branches on the 5 years old jojoba plants, B) and C) four-weeks old 1-2cm nodal segment cultured on MS supplemented with 5 μM BAP + 10 μM IAA, D) shoot elongation, and E-F) shoot multiplication.

Conclusion

In vitro nodal segment of jojoba was affected by the genotypes and growth regulator combination in the culture medium. Higher concentrations of BAP + IAA were better for shoot formation and

multiplication than BAP + NAA. The jojoba genotype 'Hada Al-Sham' showed better response to PGRs than 'Hail A', 'Medina' and 'Hail B' genotypes.

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