Plant Archives Vol. 21, No. 2, 2021 pp. 89-93 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no2.016

INFLUENCE OF AUXIN ON *IN VITRO* SECONDARY METABOLITES PRODUCTION FROM *BALANITES AEGYPTIACA* CALLUS CULTURES

Asmaa A.A. Abdel-Kareem, H.A. El-Shamy, A.K. Dawh and S.G. Gwiefel
Horticulture Department, Faculty of Agriculture, Zagazig University, Egypt

(Date of Receiving : 20-01-2021; Date of Acceptance : 24-04-2021)

ABSTRACT

The present work was conducted in order to investigate the effect of auxin type (2,4-D and NAA) and concentration (0.00, 0.25, 0.50, 1.00 and 2.00 mg/l) on *Balanites aegyptiaca* callus cultures growth and production of secondary metabolites. Obtained results demonstrated that supplementation MS medium with 2,4-D at 2.0 mg/l could enhanced and recorded the ultimate values of callus fresh weight, antioxidant activity (%), total flavonoids, total phenolic compounds and total saponins contents and yields of *Balanites aegyptiaca* L. callus.

Keywords: *Balanites aegyptiaca*, callus cultures, antioxidant activity, total flavonoids, total phenolic compounds, total saponins contents.

Abbreviation

NAA Naphthalene Acetic Acid
2,4-D 2,4-Dichlorophenoxyacetic acid

INTRODUCTION

Balanites aegyptiaca (Zygopyllaceae), commonly known as the desert dateis a widely grown desert plant with multi-use potential. It is found in most of the African continent, the Middle East, and South Asia; however, this plant remains one of the most neglected plant species (Chapagain and Wiesman, 2005). Plant contains saponins, flavonoids, alkaloids, lipids, proteins, carbohydrates and organic acids. It is traditionally used in treatment of various ailments i.e. jaundice, intestinal worm infection, wounds, malaria, syphilis, epilepsy, dysentery, constipation, diarrhea, hemorrhoid, stomach aches, asthma, and fever. It has many pharmacological activities such as; cardioprotective cum antioxidant, anthelmintic, antibacterial, antivenin, anticancer, anti-inflammatory and analgesic, antioxidant, anti-inflammatory, antinociceptive, hepatoprotective, antidiabetic, antiviral, wound healing, hypocholesterolemic andDiuretic (Chothani and Vaghasiya, 2011).

In vitro production of secondary metabolites in cell or tissue cultures has many advantages such as; year-round availability of cultures, only specific cells organs can be grown *in vitro*, secretory products can be easily purified, production of a single compound can be enhanced through the use of chemical elicitors, biotransformation or by transgene expression, the production of recombinant biopharmaceuticals such as mammalian hormones, enzymes, vaccines, and monoclonal antibodies, high consumption requires large industrial production, and secure from a natural hazardous condition (Iffat, 2019).

Auxin is an essential plant hormone that controls nearly every aspect ofa plant's life, from embryo development to organ senescence (Brumos *et al*., 2014). In *in vitro* cultures both the quality and quantity of auxin initially present in media administered during culture development have a marked effect on secondary metabolites production (Iffat, 2019).

The main objective of this study was to investigate the effect of auxin type (2,4-D and NAA) and concentration (0.00, 0.25, 0.50, 1.00 and 2.00 mg/l) on *Balanites aegyptiaca* callus cultures growth and production of secondary metabolites.

MATERIALS AND METHODS

This study was carried out in Plant Tissue Culture Laboratory of Horticulture Department, Faculty of Agriculture, Zagazig University during the period of 2017 to 2020**.**

Balanites aegyptiaca L. seeds were collocated from ten years old trees grown at North Sinai Research Station (El-Sheikh Zuwyed), Desert Research Center (DRC), Mataria, Cairo. The hard woody endocarp of the fruits was mechanically broken by light hammering to release the seed. Seeds were surface sterilized by immersion for 30 min. in fungicide solution of Rizolex (2 g/l), followed by soaking in 0.3 % mercuric chloride solution for 20 min, then transferred to sodium hypochlorite solution at 15% for 10 min. Seeds were thoroughly rinsed three times with sterile distilled water after each previous step. Sterilized seeds were cultured on MS medium (Murashige and Skoog, 1962) free from growth regulators for three weeks until the seeds were germinated and shoots were elongated. Elongated shoots were cut to obtain internode segments and leaves each alone to use as

explants for the next experiment. Internode segments (about 0.5 cm length) and leaves each alone were cultured on MS medium supplemented with different concentrations (0.0, 0.25, 0.5, 1.0 or 2.0 mg/l) of 2,4-D or NAA in order to induction of calls. Each treatment was consisted of 15 jars (60 × 120 mm), each one contained about 50 ml of medium. After three months fresh weight of callus (g), antioxidant activity (%), content of total flavonoids (mg QE/g extract),content of total phenolic compounds (mg gallic acid/g extract) and total saponins content (mg/g) were determined. Antioxidant activity (%) assay was carried out as described by Hanato *et al.* (1988).Content of total flavonoids was determined according to the method described by Ordon *et al*. (2006), while content of total phenolic compounds (TPC) was determined according to Škerget *et al*. (2005). Total saponins content (µg/ml) was estimated by colorimetric method prescribed by Goel *et al*. (2012). Total flavonoids yield (mg QE), TPC yield(mg gallic acid) and total saponins yield (mg) were estimated by multiplying content of total flavonoids (mg QE/g extract),content of total phenolic compounds (mg gallic acid/g extract) and total saponins content by callus fresh weight (g), respectively.

The cultures were placed in an air conditioned incubation room at a temperature of 25± 2 °C under 16 h/day photoperiod which provided by cool white fluorescent lamps (light intensity 2000 Lux).

Statistical analysis

The statistical layout of all experiments was simple completely randomized design. All collected data were analyzed with analysis of variance (ANOVA) procedure using the MSTAT-C Statistical Software Package (Michigan State University, 1983). Differences between means were compared by using Duncan multiple range tests (Gomez and Gomez, 1984).

RESULTS

Data presented in Table 1show that callus could not initiate on both explant types cultured on MS medium free from auxin. Comparing different concentrations of NAA and 2,4-D showed that high concentrations (1.0 and 2.0 mg/l) of 2,4-D produced the heaviest fresh weight of callus without significant difference between both concentrations. On the other side, explant type had no effect on callus fresh weight. Antioxidant activity analysis indicates that increasing of 2,4-D concentration was concomitant with gradual increase in antioxidant activity but without significant differences among medium and high concentrations (0.5, 1.0 and 2.0 mg/l) of 2,4-D in this regard. While, antioxidant activity was gradually decreased as NAA concentration was increased without significant differences among these concentrations in most cases. It is worth to mention that explant type had no significant effect on antioxidant activity.

Concerning total flavonoids content, it is clear that medium and high concentrations (0.5, 1.0 and 2.0 mg/l) of 2,4-D proved to be the best treatments to elevate this parameter, since they produced the maximum mean of total flavonoids contents (6.41,7.78 and 8.26 mg QE/g extract, respectively) without significant differences among these concentrations (Table 2). Also, 0.5 mg/l of NAA gave high content of total flavonoids (6.49mg QE/g extract) which was comparable with the above-mentioned treatments. Again explant type had no significant effect on total flavonoids content.

Total flavonoids yield followed the similar trend of total flavonoids content but with more pronounced for high concentrations (1.0 and 2.0 mg/l) of 2,4-D since they produced the highest mean of total flavonoids yield (37.47 and 46.1mg QE, respectively). There was no significant difference between both explant types in this connection.

As shown in Table 3, high concentrations (1.0 and 2.0 mg/l) of 2,4-D were the most effective treatments for enhancing mean of total phenolic compounds content, since they produced 28.64 and 31.11 mg gallic acid/g extract, respectively. It is clear that 2,4-D was more effective than NAA in this regard. Stem explants produced higher significant mean of total phenolic compounds content than leaf explant.

The same treatments (1.0 and 2.0 mg/l 2,4-D) gained the highest yield (137.91 and 173.90 mg gallic acid, respectively) of mean total phenolic compounds. There was no significant difference between both tested explants in this regard.

Concerning total saponins content (Table 4), it is clear that 2,4-D was more effective than NAA for elevating total saponins content especially at medium and high concentrations (0.5, 1.0 and 2.0 mg/l) without significant differences among these treatments. Data indicate that stem segments produced callus contained higher total saponins level than that obtained from leaf explants. Total saponins yield followed the similar trend of total saponins content. However, the highest 2,4-D concentration (2.0 mg/l) proved to be the best treatment for maximizing mean of total saponins yield, since it produced the highest significant value (18.35mg) of this parameter.

DISCUSSION

The above-mentioned results proved that 2,4-D was more effective than NAA concerning calls induction and growth. This result was previously reported by Tao *et al*. (2002) on *Citrus grandis*, Rao *et al*. (2005) on *Vigna radiate* and Gopi and Vatsala (2006) on *Gymnema sylvestre* and Masoumian *et al*. (2011) on *Hydrocotyle bonariensis*. This may be due to that 2,4-D strengthens the interaction between Aux/IAA proteins and the ubiquitin protein complex SCFTIR1, promotes the degradation of Aux/IAA and then allows the auxin response factors (ARF) activators to reactivate/activate the auxin response genes (Tan *et al*., 2007).

The results presented here indicate that, among different auxins (2,4-D and NAA) and different concentrations (0.0, 0.25, 0.5, 1.0 or 2.0 mg/l) tested, secondary metabolites of *Balanites aegyptiaca* respond most strongly to high concentrations (1.0 or 2.0 mg/l) of 2,4-D in the medium. These treatments achieved maximum contents and yields of total flavonoids and total phenolic compounds as well as the highest antioxidant activity. This efficient effect of 2,4-D compared with NAA could be interpreted in the light of Baque *et al*. (2010) and Martınez-Bonfil *et al*. (2014) results, since they revealed that the effect of auxin on secondary product formation markedly depends on the auxin types used and their concentrations.

Our results demonstrated that 2,4-D was more effective than NAA concerning callus contents of total flavonoids and

total phenolic compounds. Efficiency of 2,4-D in producing high flavonoids and phenolic compounds contents of callus was previously detected by Masoumian *et al*. (2011) on *Hydrocotyle bonariensis*, Wongsen *et al*. (2014) on sweet basil and Habibah *et al*. (2019) on *Elaeocarpus grandifloras*.

It is worth to mention that it could be observed that there was a positive correlation between high total phenol content and total flavonoid content and antioxidant activities in the above-mentioned data. This correlation was previously stated in callus cultures of *Canscora decussate* and they attributed that to the fact that both phenol and flavonoid content contributed in all the antioxidant assays tested (Kousalya and Bai, 2016).

The superiority of 2,4-D compared with NAA on *in vitro* saponins production was discovered here and early demonstrated by Jamshidi *et al*. (2014) on Fenugreek. This stimulatory effect of 2,4-D on synthesis of saponins has long been known in *P. ginseng* calluses (Furuya *et al*., 1983).

CONCLUSION

A glance on the above-mentioned results indicated that supplementation MS medium with 2,4-D at 2.0 mg/l could enhanced and recorded the maximum values of callus fresh weight, antioxidant activity (%), total flavonoids, total phenolic compounds and total saponins contents and yields of *Balanites aegyptiaca* L. callus.

Table 1 : Effect of auxin concentration and explant source on callus fresh weight (g) and antioxidant activity (%) of *Balanites aegyptiaca*

Auxin conc. (mg/l)	Callus fresh weight (g)			Antioxidant activity (%)		
	Leaf	Stem	Mean	Leaf	Stem	Mean
0.0	0.00 j	0.00 j	0.00 E	0.00 e	0.00e	0.00 E
0.25 2,4-D	3.16 efg	3.53 def	3.34 B	24.38cde	32.68 bcd	28.53 CD
0.50 2,4-D	3.56 def	4.00 cde	3.78 B	47.98 abc	52.15 ab	50.06AB
1.0 2,4-D	4.99abc	4.66 bcd	4.82 A	53.35ab	58.00 a	55.67 AB
2.0 2,4-D	6.04 a	5.20 ab	5.62 A	60.41 a	60.41 a	60.41 A
0.25 NAA	1.28 i	1.39 i	1.33 D	42.86bcd	47.98abc	45.42ABC
0.50NAA	1.63 hi	1.71 hi	1.67 CD	37.41abcd	40.41abcd	38.91BCD
1.0 NAA	2.06 ghi	2.13 ghi	2. 09 CD	31.25bcd	29.16bcd	30.20CD
2.0 NAA	2.22 ghi	2.57 fgh	2. 39 C	19.53de	24.35cde	21.94 D
Mean	2.77 A	2.79 A		35.24 A	38.34 A	

Table 2 : Effect of auxin concentration and explant source on total flavonoids content (mg QE/g extract) and yield (mg QE/g extract) of *Balanites aegyptiaca*

Auxin conc. (mg/l)	Total flavonoids content (mg QE/g extract)			Total flavonoids yield (mg QE)		
	Leaf	Stem	Mean	Leaf	Stem	Mean
0.0	0.00 f	0.00 f	0.00 D	0.00 h	0.00 h	0.00 D
0.25 2,4-D	5.50 bcde	6.20bcde	5.85 BC	17.38 def	21.88 cd	19.63 B
0.50 2,4-D	5.66bcde	7.16abcd	6.41 ABC	20.14cde	28.64 bc	24.39 B
1.0 2,4-D	7.40abc	8.16 ab	7.78 AB	36.92 a	38.02 ab	37.47A
2.0 2,4-D	7.56 abc	8.96 a	8.26 A	45.66 a	46.59 a	46.12A
0.25 NAA	4.20 e	4.66 de	4.43 C	5.37 gh	6.47gh	5.92 CD
0.50NAA	6.23 bcde	6.76 abcde	6.49 ABC	10.15 efg	11.5 defg	10.82 C
1.0 NAA	5.23 cde	5.43cde	5.33 C	10.77efg	11.56efg	11.16 C
2.0 NAA	4.50 de	4.53 de	4.51 C	9.99 fgh	11.6defg	10. 79 C
Mean	5.14 A	5.76 A		17.37A	19.58 A	

Table 3 : Effect of auxin concentration and explant source on TPC* content (mg gallic acid/g extract) and yield (mg gallic acid) of *Balanites aegyptiaca*

Auxin conc. (mg/l)	TPC content (mg gallic acid/g extract)			TPC yield (mg gallic acid)		
	Leaf	Stem	Mean	Leaf	Stem	Mean
0.0	0.00 j	0.00 j	0.00 E	0.00 g	0.00 g	0.00 D
0.25 2,4-D	23.16efg	26.23cde	24.69 C	73.18 de	92.59cd	82.88 B
0.50 2,4-D	25.10def	29.90abc	27.50 B	89.35 cd	119.6bc	104.47 B
1.0 2,4-D	26.86bcde	30.43ab	28.64AB	134.03ab	141.80ab	137.91 A
2.0 2,4-D	28.83bcd	33.40a	31.11 A	174.13a	173.68a	173.90 A
0.25 NAA	18.23 hi	21.20 fghi	19.71D	23.33 fg	29.46fg	26.39 C
0.50NAA	21.43fgh	19.40ghi	20.41 D	34.93f	33.17fg	34.05 C
1.0 NAA	20.10ghi	19.50ghi	19.80 D	41.40 ef	41.53ef	41.46C
2.0 NAA	17.23i	20.46 ghi	18.84 D	38.25 f	52.58 ef	45.41 C
Mean	20.10B	22.28A		67.62A	76.04 A	

*Total phenolic compounds

Table 4 : Effect of auxin concentration and explant source on total saponins content (mg/g) and yield (mg) of *Balanites aegyptiaca*

Auxin conc. (mg/l)	Total saponins content (mg/g)			Total saponins yield (mg)		
	Leaf	Stem	Mean	Leaf	Stem	Mean
0.0	0.00 g	0.00 g	0.0 F	0.00 i	0.00 i	0.00 F
0.25 2,4-D	2.97 abcd	3.07 abcd	3.02 BCD	9.39 def	10.87 de	10.13 C
0.50 2,4-D	3.07 abcd	3.19 abc	3.13 ABC	10.93 de	12.78 cd	11.85 C
1.0 2,4-D	3.15 abcd	3.22 ab	3.19 AB	15.75 bc	15.04 b	15.40 B
2.0 2,4-D	3.22 ab	3.31 a	3.27 A	19.47 a	17.24 bc	18.35 A
0.25 NAA	1.96 f	2.34 e	2.15 E	2.52 hi	3.25 ghi	2.88 E
0.50NAA	1.97 f	2.35 e	2.16 E	3.22 ghi	4.02 ghi	3.62 E
1.0 NAA	2.83 d	2.88 bcd	2.86 D	5.84 fgh	6.14 fg	5.99 D
2.0 NAA	2.85 cd	2.97 bcd	2.91 CD	6.33 fg	7.63 ef	6.98 D
Mean	2.45 B	2.59 A		8.16 A	8.55 A	

REFERENCES

Baque, M.d.A.; Hahn, E. and Paek, K. (2010). Growth, secondary metabolite production and antioxidant enzyme response of *Morinda citrifolia* adventitious root as affected by auxin and cytokinin. *Plant Biotechnol. Rep*., 4: 109–116.

Brumos, J.; Alonso, J.M. and Stepanova, A.N. (2014).Genetic aspects of auxin biosynthesis and its regulation. *Physiologia Plantarum,* 151 (1): 3-12.

Chapagain, B. and Wiesman, Z. (2005).Variation in diosgenin level in seed kernels among different provenances of *Balanites aegyptiaca* Del (*Zygophyllaceae*) and its correlation with oil content. *African Journal of Biotechnology* 4 (11): 1209-1213.

Chothani, D.L. and Vaghasiya, H.U. (2011). A review on *Balanites aegyptiaca* Del (desert date): phytochemical constituents, traditional uses, and pharmacological activity. *Pharmacogn. Rev*. 5 (9): 55–62.

Furuya, T.; Yoshikawa, T.; Ishii, T. and Kajii, K. (1983). Effects of auxins on growth and saponin production in Callus Cultures of *Panax ginseng*. *Planta Med.,* 47: 183–187.

Goel, N.; Sirohi, S.K. and Dwivedi, J. (2012). Estimation of total saponins and evaluate their effect on *in vitro* methanogenesis and rumen fermentation pattern in wheat straw based diet. *Journal of Advanced Veterinary Research,* 2: 120-126.

Gomez, K.A. and Gomez, A.A. (1984). Statistical Procedures for Agricultural Research. Wiley, New York, USA.

Gopi, C. and Vatsala, T.M. (2006). *In vitro* studies on effects of plant growth regulators on callus and suspension culture biomass yield from *Gymnema sylvestre R.Br. African Journal of Biotechnology,* 5(12): 1215-1219.

Habibah, N.A.; Nugrahaningsih, W.H.; Musafa, F.; Rostriana, Y.; Mukhtar, K.; Wijawati, N. and Anggraito, Y.U. (2020). Bioactive compounds from callus culture of *Elaeocarpus grandifloras*. *Journal of Physics: Conference Series,* 1567: 1-4.

Hanato, T.; Kagawa, H.; Yasuhara, T. and Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *Chem. Pharm. Bull*. 36: 2090–2097.

Iffat, W. (2019). Effect of tissue culture conditions on production of secondary metabolites. *International Journal of Advances in Pharmacy Medicine and Bioallied Sciences,* 7(1): 10-16.

Jamshidi, S.; Lahouti, M. and Ganjeali, A. (2014). Assessment of callus growth and bio-production of diosgenin in callus culture of *Trigonella foenum-graecum* L. *Bull.Env. Pharmacol. Life Sci*., 3: 191-198.

Kousalya, L. and Bai, V.N. (2016). Effect of growth regulators on rapid micropropagation and antioxidant activity of *Canscora decussata* (Roxb.) Roem. & Schult. – A threatened medicinal plant. *Asian Pacific Journal of Reproduction,* 5(2): 161–170.

Martınez-Bonfil, B.P.; Cruz-Hernandez, A.; Lopez-Laredo, A.R.; Trejo-Tapia, G. and Trejo-Espino, J.L. (2014). Effects of culture medium and auxins on growth of adventitious root cultures of *Cupheaa equipetala* Cav. and their ability to produce antioxidant compounds. *Plant Cell Tiss. & Organ Cult*. 118: 401–408.

Masoumian, M.; Arbakariya, A.; Syahida, A. and Maziah, M. (2011). Flavonoids production in *Hydrocotyle bonariensis* callus tissues. Journal of Medicinal, *Plants Research* 5(9): 1564-1574.

Michigan State University (1983). MSTAT-C Micro Computer Statistical Programe, Version 2.Michigan State University, East Lansing.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum*, 15: 473- 497.

Ordon, J.D.; Gomez, M.A. and Vattuone, M.I. (2006). Antioxidant activities of *Sechiumedule* (Jacq.) Swartz extracts. *Food Chem*. 97: 452-458.

Rao, S.; Patil, P. and Kaviraj, C.P. (2005). Callus induction and organogenesis from various explants in *Vigna radiate* (L.) Wilczek. *Indian Journal of Biotechnology,* 4: 556-560.

Škerget, M.; Kotnik, P.; Hadolin, M.; Rižner-Hraš, A.; Simonič, M. and Knez, Ž. (2005). Phenols, pro anthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem*., 89: 191–198.

Tan, X.; Calderon-Villalobos, L.I.A.; Sharon, M.; Zheng, C.; Robinson, C.V.; Estelle, M. and Zheng, N. (2007). Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature,* 446: 640–645.

Tao, H.; Shaolin, P.; Gaofeng, D.; Lanying, Z. and Gengguang, L. (2002). Plant regeneration from leaf-derived callus in *Citrus grandis* (pummelo): Effects of auxins in callus induction medium. *Plant Cell, Tissue and Organ Culture,* 69: 141–146.

Wongsen, W.; Bodhipadma, K.; Noichinda, S. and Leung, D.W.M. (2015). Influence of different 2,4-D concentrations on antioxidant contents and activities in sweet basil leaf-derived callus during proliferation. *International Food Research Journal,* 22(2): 638-643.