IJANS International Journal of Agricultural and Natural Sciences
Uluslararası Tarım ve Doğa Bilimleri Dergisi
E-ISSN: 2651-3617 1(1): 29-32, 2018

In vitro Carrot (*Daucus carota* L.) Regeneration: A Study on the Use of 2,4-D and Activated Charcoal

Tugce OZSAN[1] M. Gokce KANMAZ[1] A. Naci ONUS[1]*
[1]Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya, TURKEY

*Corresponding Author
E-mail:onus@akdeniz.edu.tr

Abstract
Regeneration can be provided with *in vitro* techniques in carrot (*Daucus carota* L.), but the response to regeneration depends on a number of factors, such as genotype, explant type, growth media, plant growth regulators and their concentrations. In the present study, Nanco and Maestro seeds were germinated, then three different types of explants (cotyledon, hypocotyl, true leaf) cultured in Murashige & Skoog (MS) media, supplemented with activated charcoal (1,0 g/l) and various doses of 2,4-Dichlorophenoxyacetic acid (2,4-D). Obtained results showed that Nanco cultivar had responded much better to *in vitro* regeneration than Maestro. While explant types were evaluated, it was recorded that hypocotyls and true leaves were more responsive than cotyledons. In terms of media, MS + 2,0 mg/l 2,4-D + 1,0 g/l activated charcoal was found to be the most successful.

Keywords: Carrot, *Daucus carota* L., *in vitro*, regeneration, 2,4-D, activated charcoal

INTRODUCTION

Carrot (*Daucus carota* L.) is a valuable plant variety and important member of the *Apiaceae* family. The carrot plant, which has a very high economic value, is among the biennial vegetables consumed. Many species produce and accumulate vitamins, carotenoids and anthocyanins in their storage roots [1], [2], [3] as well as carrot. Carrots are especially rich in vitamin A and have carotene and anthocyanins [4]. Having important bioactive components makes carrot an important crop for human health. It also has the potential to be used as a model plant in genetic studies [5], [6].

The tissue culture technique, which is a clonal propagation method, first provides sterilization of different plant tissue parts (explants). Then, aseptic, artificial media are used to culture and new tissue, plant or plant parts are tried to be obtained in controlled and sterile conditions. Tissue culture techniques can be used to develop new varieties, to create genetic diversity and the protect plants valuable but difficult to propagate.

The most commonly used plant growth regulators for growth and morphogenesis *in vitro* cultures are auxins and cytokinins which can be obtained both naturally and artificially. Different regenerations can be achieved by changing the concentrations of auxin and cytokinin in media and using different plant tissues. One of the most commonly used synthetic auxins is 2,4-Dichlorophenoxyacetic acid (2,4-D) [7].

Activated charcoal has many uses on *in vitro* studies. It is usually added to the media between 0.2 and 3.0%. It then has many effects such as on microbial growth, callus and protoplast cultures, haploid studies, rooting, shoot formation and prolongation. It also has ability to inhibit the substances preventing the growth in media [8].

In this study, 3 different explants (cotyledon, hypocotyl, true leaf) from Nanco and Maestro carrot cultivars were used for *in vitro* studies which was conducted to reveal the *in vitro* regeneration effects of 2,4-D (1,0 mg/l and 2,0 mg/l) and activated charcoal (1,0 g/l).

MATERIALS AND METHODS

The study was carried out in the Tissue Culture Laboratory of the Department of Horticulture, Faculty of Agriculture, Akdeniz University.

Plant Material

Nanco and Maestro carrot seeds were used as plant material.

Sterilization

Surface sterilization of seeds was carried out as down stated:

i. Seeds were subjected to surface sterilization with 15% sodium hypochlorite solution for 15 minutes,
ii. Then they were left in 70% alcohol for 15 seconds.
iii. Seeds were rinsed 3 times with sterile distilled water.

After all these treatments, 15 seeds were cultured in prepared jar.

Media Preparation

In present study Murashige & Skoog (MS) [9] basal medium with no plant growth regulators was used for *in vitro* seed germination.

For determining regeneration capabilities, 5 different nutrient media combinations, including control medium, were tested as shown in Table 1. For media preparation Murashige & Skoog (MS) basal medium was used. Media were supplemented with different concentrations of 2,4-Dichlorophenoxy acetic acid (1,0 and 2,0 mg/l). Two other media (number IV and V) 1,0 g/l activated charcoal were also used. All media, including control medium, were prepared with 30,0 g/l sucrose as a carbohydrate source and solidified by using 6,0 g/l agar. Media pH was adjusted to 5.8 using NaOH and HCl solutions. The prepared media were autoclaved at 121°C and 1 atmosphere for 15 minutes.

Cultivation

Under sterile conditions, three types of explants (cotyledon, hypocotyl, true leaf) were taken from *in vitro* germinated seedlings. Afterwards each pieces were inoculated in each petri dishes containing prepared media.

Incubation

The cultivated seeds were kept at 25 °C for 3 days at constant dark conditions, and then transferred to the growth room with 16/8 hours photoperiod and 3000 lux illumination at 24 ± 2 °C. Cotyledon, hypocotyl and true leaf parts of these *in vitro* obtained plants were taken as explants and cultured in each prepared medium. And they also were kept at same conditions as mentioned above.

Observations

Regeneration capabilities of three types of explants belong to two different genotypes were observed and recorded.

Table 1. The Nutrient Media Used in The Study

Media Codes	Basal Media	2,4 – D (mg/l)	AC (g/l)	Sucrose (g/l)	Agar (g/l)
I*	MS	-	-	30,0	6,0
II	MS	1,0	-	30,0	6,0
III	MS	2,0	-	30,0	6,0
IV	MS	1,0	1,0	30,0	6,0
V	MS	2,0	1,0	30,0	6,0

*Control medium; AC: Activated charcoal

RESULTS AND DISCUSSION

Carrot seeds of Nanco and Maestro were germinated. Following the germination, three different explants (cotyledon, hypocotyl, true leaf) were taken from the plants. *In vitro* regeneration responds of different explants were recorded.

Seeds sown in MS0 medium started to germinate at the end of 3rd day in medium and formed plantlet (Fig. 1, Fig. 2).

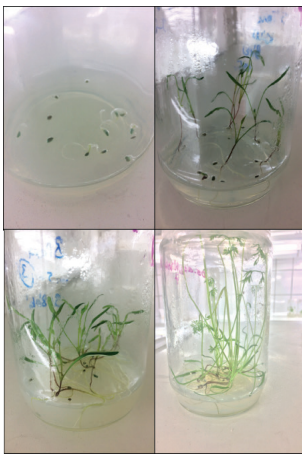

Fig. 1. Developments of Nanco seeds

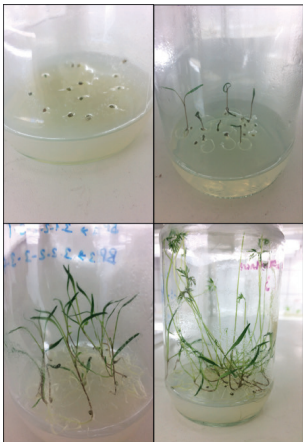

Fig. 2. Developments of Maestro seeds

After plantlet formation, cotyledon, hypocotyl and true leaf were taken and used as explants. Experimental results revealed that there were differences among explants in terms of responding to *in vitro* regeneration. All explants formed the whole plant within forty days in media with no activated charcoal but 2,4-D.

As reported by [10], direct organogenesis in the presence of benzylaminopurine is encouraged within four weeks, whereas organogenesis in the presence of 2,4-D can be delayed up to sixty days. Finding of present study about the effect of 2,4-D on *in vitro* regeneration is in agreement with it.

Concerning the cultivars and explant types, plantlets were obtained from the hypocotyl explants in control medium for cultivar Maestro, while plantlets were obtained from hypocotyl and true leaf explants for cultivar Nanco (Fig. 3).

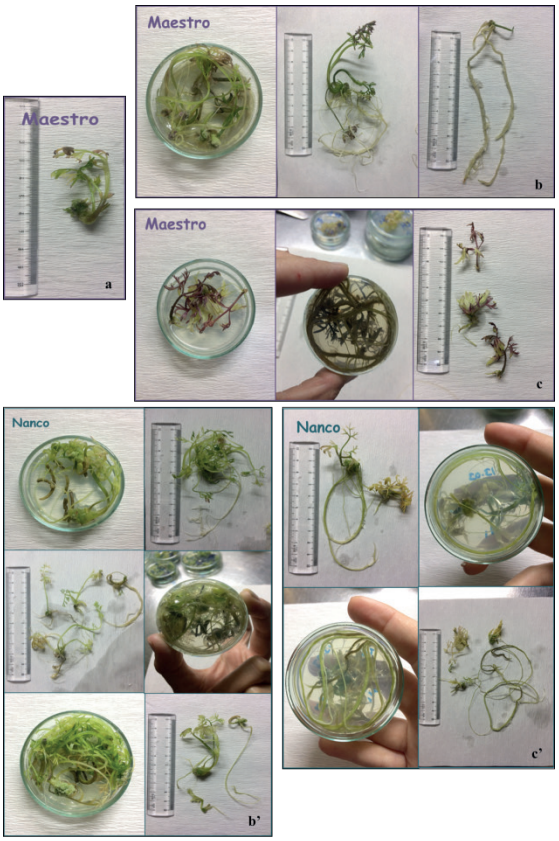

Fig. 3. The emerging developments in Medium number I (control medium); Maestro a. Cotyledon, b. Hypocotyl, c. True leaf; Nanco b': Hypocotyl, c': True leaf

Concerning the other media combinations; II (MS + 1,0 mg/l 2,4-D) and III (MS + 2,0 mg/l 2,4-D), all explants formed callus for both cultivars. In media number II and III, there was no activated charcoal but 2,4-D induced the callus formation.

Regarding the medium no IV (1,0 mg/l 2,4-D + 1,0 g/l activated charcoal) cotyledon explants provided callus formation for Maestro cultivar, but there was no respond for cultivar Nanco. On the other hand hypocotyl explants resulted with plantlet formation for both cultivars. In addition to these, Nanco cultivar had plantlet formation for true leaf explants in medium IV (Fig. 4). As a matter of fact, 2,4-D, which is considered as one of the most important auxins in previous studies, has been stated as a promoter of callus formation [10], [11], [12], [13], [14].

If we evaluate the medium no V (2,0 mg/l 2,4-D + 1,0 g/l activated charcoal) plantlets were obtained from cotyledon explants of Nanco, while there was no plantlet formation for

Maestro's cotyledon explants. On the other hand, in medium V, hypocotyl explants of both cultivars provided well developed plantlets. Regarding true leaf explants in medium V, while plantlets were obtained for cultivar Nanco, there was only root formation for Maestro (Fig. 5).

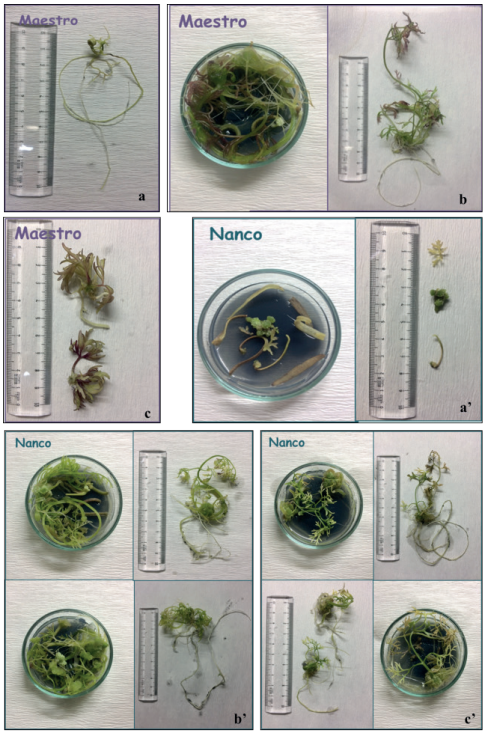

Fig. 4. The emerging developments in Medium number IV; Maestro a. Cotyledon, b. Hypocotyl, c. True leaf; Nanco a': Cotyledon, b': Hypocotyl, c': True leaf

In Nanco, true leaf explants formed plants, while only root formation was observed in Maestro. Activated charcoal,which we observed as an inducer for regeneration, has a beneficial effect on cell growth and development, as stated in previous studies [8], [15].

In another previous study, it was reported that 2,4-D was adsorbed 99.5% within 5 days of adding the activated charcoal to the liquid medium [8], [16], clearly showing the relation between activated charcoal and 2,4-D.

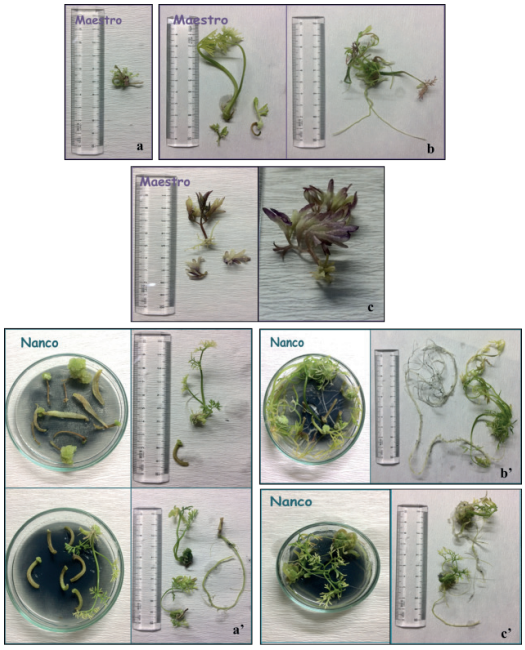

Fig. 5. The emerging developments in Medium number V; Maestro a. Cotyledon, b. Hypocotyl, c. True leaf; Nanco a': Cotyledon, b': Hypocotyl, c': True leaf

CONCLUSION

In this study, seeds of two different carrot varieties were sown under sterile and controlled conditions. The reactions of cotyledon, hypocotyl and true leaf explants to various media have been studied. Callus formation was observed in media, not supplemented with activated charcoal. The responses of the hypocotyl explants were more favorable and also developments of the other two types of explants were recorded. It was observed that the use of activated charcoal in the study effected plant regeneration positively. We believe that trying different concentrations of both 2,4-D and activated charcoal can be useful for further carrot regeneration studies.

REFERENCES

[1] G.L. Wang, Z.S. Xu, F. Wang, M.Y. Li, G.F. Tan, A.S. Xiong, Regulation of ascorbic acid biosynthesis and recycling during root development in carrot (*Daucus carota* L.). Plant Physiol Biochem 94 (2015), pp. 10–18.

[2] C.H. Luby, H.A. Maeda, I.L. Goldman, Genetic and phenological variation of tocochromanol (vitamin E) content in wild (*Daucus carota* L. var. *carota*) and domesticated carrot (*D. carota* L. var. *sativa*). Hortic Res, 1 (2014), pp. 14015.

[3] H. Yuan, J. Zhang, D. Nageswaran, L. Li Carotenoid metabolism and regulation in horticultural crops. Hortic Res, 2 (2015), pp. 15036.

[4] U. Dereli, Siyah Havuç Suyu Konsantresi Üretimi ve Depolanması Sürecinde Fenolik Maddelerdeki Değişimler ve Bu Değişimlerin Antioksidan Aktivite ile İlişkisi. Yüksek Lisans Tezi. Ankara Üniversitesi, Fen Bilimleri Enstitüsü (2010).

[5] C. Grebenstein, S.P. Kos, T.J. de Jong, W.L.M. Tamis, G.R. de Snoo, Morphological markers for the detection of introgression from cultivated into wild carrot (*Daucus carota* L.) reveal dominant domestication traits. Plant Biol 15 (2013), pp. 531–540.

[6] M. Jourdan, S. Gagné, C. Dubois-Laurent, M. Ma-

ghraoui, S. Huet, A. Suel, L. Hamama, M. Briard, D. Peltier, E. Geoffriau, Carotenoid content and root color of cultivated carrot: a candidate-gene association study using an original broad unstructured population. PLoS One, 10 (2015), pp. e0116674.

[7] E.F. George, M.A. Hall and G.J. De Klerk,. Plant Growth Regulators I: Introduction; Auxins, their Analogues and Inhibitors. Plant Propagation by Tissue Culture 3rd Edition, Vol.1 The Background, Springer, (2008), pp. 501.

[8] D. Thomas, The role of activated charcoal in plant tissue culture. Biotechnology Advances 26 (2008), pp. 618–631.

[9] T. Murashige, F. Skoog, A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant, 15 (1962), pp. 473–97.

[10] N. Anupama, D.H. Tejavathi, and V.R. Devaraj, *In vitro* Regeneration of Cultivars of Colocasia esculenta (L.) Schott. Int. J. Pure App. Biosci., 6 (1) (2018), pp. 920-926. DOI: http://dx.doi.org/10.18782/2320-7051.6101.

[11] E.F. George and P.D. Sherrington, Plant propagation by tissue culture: Handbook and Directory of commercial laboratories, London, Exegetics Ltd. (1984).

[12] M.Y. Zheng and C.F. Konzak, Effect of 2,4-dichlorophenoxyacetic acid on callus induction and plant regeneration in anther culture of wheat (*Triticum aestivum* L.). Plant Cell Rep, 19 (1999), pp. 69–73.

[13] S.D. Novak, L.J. Luna, R.N. Gamage, Role of auxin in orchid development. Plant Signal Behav 9 (10) (2014), pp. e972277. https://doi.org/10.4161/psb.32169.

[14] M.R. Abdollahi and S. Rashidi, Production and conversion of haploid embryos in chickpea (*Cicer arietinum* L.) anther cultures using high 2,4-D and silver nitrate containing media. Plant Cell, Tissue and Organ Culture (PCTOC) 133 (2018), pp. 39–49 https://doi.org/10.1007/s11240-017-1359-4.

[15] M.J. Pan, J. Van Staden The use of charcoal in *in vitro* culture—a review. Plant Growth Regul; 26 (1998), pp. 155–63.

[16] A. Ebert and H.F. Taylor, Assessment of the changes of 2,4- dichlorophenoxyacetic acid concentrations in plant tissue culture media in the presence of activated charcoal. Plant Cell Tissue Organ Cult, 20 (1990), pp. 165–72.