The Journal of Animal & Plant Sciences, 27(5): 2017, Page: 1671-1677
ISSN: 1018-7081

EFFECTS OF DIFFERENT CONCENTRATIONS OF BAP AND NAA ON MICROPROPAGATION OF *Crambe orientalis* L. var. *orientalis* L.

Y. Ceylan[1], F.A. Özdemir[2] and A. S. Bülbül[1*]

[1]Department of Molecular Biology and Genetics, Faculty of Science, Bartin University, 74000, Bartin, Turkey
[2]Department of Molecular Biology and Genetics, Faculty of Science and Art, Bingol University, 12000, Bingol, Turkey
*Correspondence: asavasbulbul@gmail.com

ABSTRACT

Crambe L. is a species of the Brassicaceae family. It is an important industrial oil plant with high erusic acid content. The objective of this study was to develop an efficient micropropagation protocol for *Crambe orientalis* L. var. *orientalis* L. species using different concentrations of BAP (6 - benzylaminopurine)–NAA (α - naphthalene acetic acid) hormone combinations. Seeds of *Crambe orientalis* var. *orientalis* were germinated *in vitro*. Cotyledon node and hypocotyl parts from fourteen-days seedlings were used as explant sources. The explants were cultured on MS media containing 0.25, 0.50, 1.00 and 2.00 mg/L BAP and 0, 0.25 and 0.50 mg/L NAA. Effects of hormone concentrations on mean callus regeneration percentage (%), mean shoot regeneration percentage (%), mean number of shoots per explant and mean length of shoots were determined. Callus formation and shoot regeneration resulted in all hormone concentrations used for the hypocotyl and cotyledon explants in our study. All explants were rooted on MS media containing 1.00 and 2.00 (mg/L) NAA.

Key words: BAP, Brassicaceae, callus, *Crambe orientalis* L. var. *orientalis* L., micropropagation, NAA.

INTRODUCTION

Brassicaceae family consists of 350 genera and 4000 species in the world. In addition, 85 genera and 515 species grow in Turkey. Most species of the Brassicaceae family are densely distributed in the mild and especially chilly districts of Northern Hemisphere. Besides, it is seen on Mediterranean region in addition to Southwest and Central Asia. *Crambe* L. species which is annual and perennial have been used as oil crop plant (Grombacher *et al*., 1993; Yang *et al*., 2005). There are more than 30 species in the world (Durak, 1999; Knights, 2002). The emerging district of crambe is known as the Mediterranean region and Iran-Turan province of Southern and Western Asia. It is able to grow both in winter and spring due to its resistance to adverse conditions. Crambe has spread widely from Asia to Western Europa thanks to its high adaptation capacity (Köybaşı, 2008). Crambe can grow in shade or semi - shade soils and poor nutrient soil. It can withstand cold temperatures down to -20°C. The tolerance of crambe to salt is similar to that of wheat (Glaser, 1996; Enders *et al*., 2013). Crambe has greater resistance against warmth and aridity during the growing season in comparison with canola and it also has a yield that is much more stable (Knights, 2002; Lalas *et al*., 2012). Crambe grows best on clay soil medium however; it can also be grown in sandy soil (Seyis *et al*., 2013). Crambe also has greater resistance against drought in comparison canola, corn, mustard and soy beans (Enders *et al*., 2013). Water and irrigation problems due to global warming promote scientific studies to produce plants that are resistant against drought. These studies have shown that crambe species are extremely important plants (Wang *et al*., 2000). Crambe oil is a long-fatty acid and contains more 10 - 15% erusic acid than other industrial seed oils (Yang *et al*., 2005; Li *et al*., 2012). The fat content of crambe fruit and seed is 17.5% and 30 - 38% respectively. The upper parts of the plant as leaves and stem contain comarin, vitamin C and β - carotene (Okhunov *et al*., 2011, Okhunov *et al*., 2012). The remainder following fat extraction from seeds is rich in protein content and is utilized as animal fodder. The protein - amino acid composition in animal meal indicates its high nutritional value (Gastaldi *et al*., 1998; Stolarski *et al*., 2013). The dry weight of glume harvested with the seed is rich in cellulose at a percentage of 25% and hence can be used in the paper industry (Tittonel, 1995; Tutuş *et al*., 2010). Moreover, crambe is an alternative plant for producing biodiesel. As a result of issues about climate change and fuel reservation, oil plants attract attention for reducing the threat of global warming and for eliminating air pollution caused by fuels in recent years (Seyis *et al*., 2013).

The objective of this study was to develop an effective regeneration protocol for the hypocotyl and cotyledon node explants of *Crambe orientalis* var. *orientalis* using different concentrations of BAP - NAA combinations.

MATERIALS AND METHODS

Seeds of *Crambe orientalis* L. var. *orientalis* L., collected from its natural habitat were used as plant material in this study. The seeds were procured from Field Crops Central Research Institute. All used media in this study were sterilized at 1.5 atm for twenty minutes at 120°C in an autoclave (Nüve). The sterilization of other equipment such as petri dish, Erlenmeyer flask, beaker, forceps, scalpels were carried out at 160°C for four hours in Pasteur's oven (Mipro). The seeds were treated with commercial bleach (ACE - Turkey, 5% NaOCl) for twenty minutes after which surface-sterilized seeds were rinsed with sterilized dH2O thrice for three minutes. They were then cultured on agar solidified MS medium (Murashige and Skoog, 1962) containing 3% sucrose. The seeds began to germinate after fourteen days. Hypocotyl and cotyledon nodes from germination seeds are used as explants under aseptic conditions. Hypocotyl and cotyledon node explants were cultured on MS medium that contains 0.25, 0.50, 1.00 and 2.00 mg/L BAP and 0, 0.25 and 0.50 mg/L NAA (twelve different combinations). In conclusion, shoots were transferred to MS medium which contains 0.50, 1.00 and 2.00 mg/L NAA to root. The pH values of all used media were adjusted to pH 5.8 ± 1 by using 1 N NaOH and HCl. All studies were performed in a growth cabinet (Aralab) with a 16 h photoperiod under cool-light (500 µmolm-2s-1) at 24 ± 1°C.

Replications contain 10 explants each of which has been replicated three times. One-way analysis of variance was applied on experimental data in addition to post hoc Tukey's b test has via IBM SPSS for Windows v. 20.

RESULTS

In this study, the cotyledon nodes and hypocotyls of fourteen days old seedlings of *in vitro* germinated *Crambe orientalis* var. *orientalis* seeds were used as explant. The effects of the used hormone concentrations on callus induction (%), shoot induction (%), mean shoot number per explant and mean shoot length were studied. The effects of the hormone concentrations used on hypocotyls and cotyledon nodes have been given respectively in Table 1 and Table 2.

Callus formation from hypocotyl explants was observed for all hormone combinations. The rate of callus formation was between 67.87% and 1.22% (Table 1). The highest callus formation was observed on H9 medium with a rate of 67.87% (Figure 1a). The lowest callus formation resulted on H4 medium with a rate of 1.22%.

Shoot formation of hypocotyl explants was observed on all hormone combinations. The mean shoot formation was between 44.49% and 95.67% (Table 1). The highest mean shoot formation was detected on H7 medium with a rate of 95.67% (Figure 1b). The lowest mean shoot formation was observed on H9 medium with a rate of 44.49%.

The mean shoot number per explant varied between 27.34 ± 0.69 and 6.02 ± 0.68. The highest shoot number was 27.34 ± 0.69 on H7 medium (Figure 1c), whereas the lowest shoot number was 6.02 ± 0.68 on H12 medium. Reducing NAA hormone concentration from 0.50 mg/L to 0.25 mg/L caused a prominent increase in the mean shoot number per explant.

The mean shoot length was between 7.54 ± 0.62 cm and 3.12 ± 1.24 cm (Table 1). The longest shoots occurred as 7.54 ± 0.62 cm on H3 medium (Figure 1d), the shortest was 3.12 ± 1.24 cm on H1 medium.

Callus formation of cotyledon nodes was observed for all hormone combinations. The callus formation was between 51.64% and 1.76% (Table 2). The highest mean callus formation was detected on CN9 medium with a rate of 51.64% (Figure 2a). The lowest mean callus formation was on CN4 medium with a rate of 1.22%.

Whereas the mean callus formation increased due to increasing NAA concentration, it decreased due to increasing BAP concentration for both explants.

Shoot formation of cotyledon node explants were observed on all hormone combinations. The mean shoot formation was between 49.71% and 73.17% (Table 2). The highest mean shoot formation was observed on CN7 medium with a rate of 73.17% (Figure 2b). The lowest mean shoot formation was observed on CN9 medium with a rate of 49.71%.

The mean shoot number per explant varied between 18.14 ± 1.36 and 6.12 ± 0.98. The highest shoot number was 18.14 ± 1.36 on CN8 medium (Figure 2c), the lowest was 6.12 ± 0.98 on CN9 medium. Reducing NAA hormone concentration from 0.50 mg/L to 0.25 mg/L resulted in a prominent increase in the mean shoot number per explant.

The mean shoot length varied between 5.64 ± 2.01 cm and 2.66 ± 1.66 cm (Table 2). The longest was 5.64 ± 2.01 cm on CN4 medium (Figure 2d), whereas the shortest was 2.66 ± 1.66 cm on CN2.

Hypocotyl explant is a more productive explant in terms of forming shoot compared to cotyledon node explant.

Regenerating shoots were rooted on MS medium containing 0.50, 1.00 and 2.00 mg/L NAA (Figure 3a, 3b and 3c). The best rooting frequency has been found with 41.32% on MS medium containing 2.00 mg/L NAA (Figure 3c).

Table 1. The effects of different BAP and NAA hormone concentrations on hypocotyl explant.

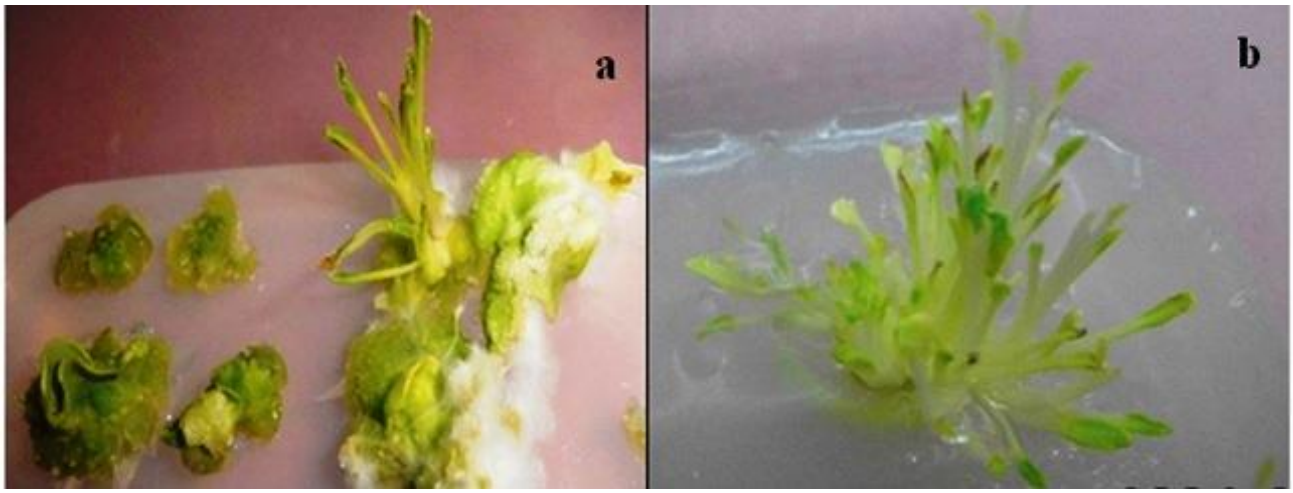
MS medium	Hormone		The mean callus formation (%)	The mean shoot formation (%)	The mean shoot number per explant	The mean shoot length
	BAP (mg/L)	NAA (mg/L)				
H1	0.25	0.0	5.61^{h}	62.46^{h}	16.23±0.72^{e}	3.12±1.24^{f}
H2	0.50	0.0	3.49^{i}	69.93^{g}	19.83±1.01^{d}	5.11±2.21^{d}
H3	1.00	0.0	1.62^{j}	74.19^{f}	20.01±0.66^{d}	7.54±0.62^{a}
H4	2.00	0.0	1.22^{j}	78.42^{d}	22.29±1.73^{c}	6.96±0.86^{b}
H5	0.25	0.25	43.83^{c}	75.66^{e}	20.61±2.42^{d}	6.13±1.62^{c}
H6	0.50	0.25	36.99^{d}	83.73^{c}	24.66±1.52^{b}	4.22±2.42^{e}
H7	1.00	0.25	21.21^{f}	95.67^{a}	27.34±0.69^{a}	7.25±0.74^{a}
H8	2.00	0.25	13.44^{g}	88.11^{b}	25.03±2.02^{b}	5.99±2.34^{c}
H9	0.25	0.50	67.87^{a}	44.49^{l}	10.20±1.26^{b}	6.00±0.98^{c}
H10	0.50	0.50	52.69^{b}	47.34^{k}	9.96±2.00^{f}	5.03±1.36^{d}
H11	1.00	0.50	36.16^{d}	58.29^{j}	9.77±1.44^{f}	5.22±1.46^{d}
H12	2.00	0.50	32.91^{e}	61.25^{i}	6.02±0.68^{g}	6.23±0.72^{c}

Means ± standard error within a column followed by superscripts are statistically significant ($P \leq 0.01$).

Table 2: The effects of different BAP and NAA hormone concentrations on cotyledon node explant.

MS medium	Hormone		The mean callus formation (%)	The mean shoot formation (%)	The mean shoot number per explant	The mean shoot length
	BAP (mg/L)	NAA (mg/L)				
CN1	0.25	0.0	11.19^{i}	59.64^{g}	9.36±1.62^{g}	5.23±0.54^{a}
CN2	0.50	0.0	6.16^{j}	66.03^{f}	11.16±0.98^{f}	2.66±1.66^{f}
CN3	1.00	0.0	3.48^{k}	67.29^{e}	15.63±1.10^{c}	3.12±0.86^{e}
CN4	2.00	0.0	1.76^{l}	70.02^{c}	17.61±2.01^{b}	5.64±2.01^{a}
CN5	0.25	0.25	41.25^{c}	68.16^{d}	14.09±1.22^{e}	4.10±1.01^{d}
CN6	0.50	0.25	36.93^{d}	71.15^{b}	15.13±1.66^{d}	4.99±0.66^{b}
CN7	1.00	0.25	27.33^{f}	73.17^{a}	17.95±2.41^{b}	4.88±0.42^{c}
CN8	2.00	0.25	19.26^{h}	72.96^{a}	18.14±1.36^{a}	5.11±1.08^{a}
CN9	0.25	0.50	51.64^{a}	49.71^{k}	6.12±0.98^{j}	4.76±0.59^{c}
CN10	0.50	0.50	43.65^{b}	52.73^{j}	8.61±1.63^{i}	5.02±1.21^{b}
CN11	1.00	0.50	31.29^{e}	55.19^{i}	9.03±0.92^{h}	4.26±0.64^{d}
CN12	2.00	0.50	23.34^{g}	57.44^{h}	9.69±1.06^{g}	3.18±0.71^{e}

Means ± standard error within a column followed by superscripts are statistically significant ($P \leq 0.01$)



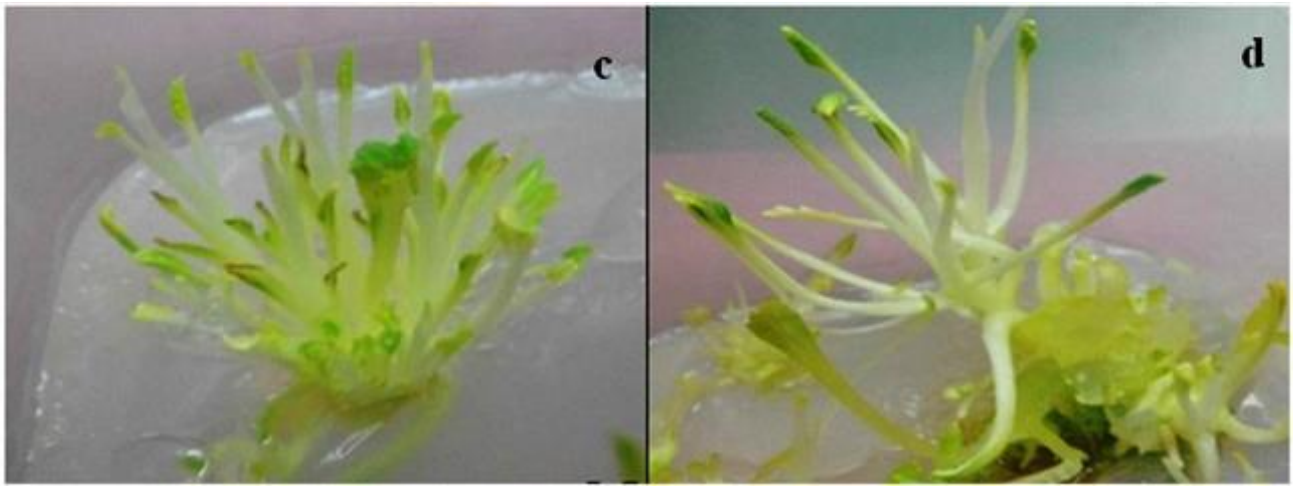


Figure 1. For hypocotyl explant (a) maximum callus formation on H9 medium, (b) maximum regeneration shoot rate on H7 medium, (c) maximum number of shoot per explant on H7 medium (d) the longest shoot on H3 medium.

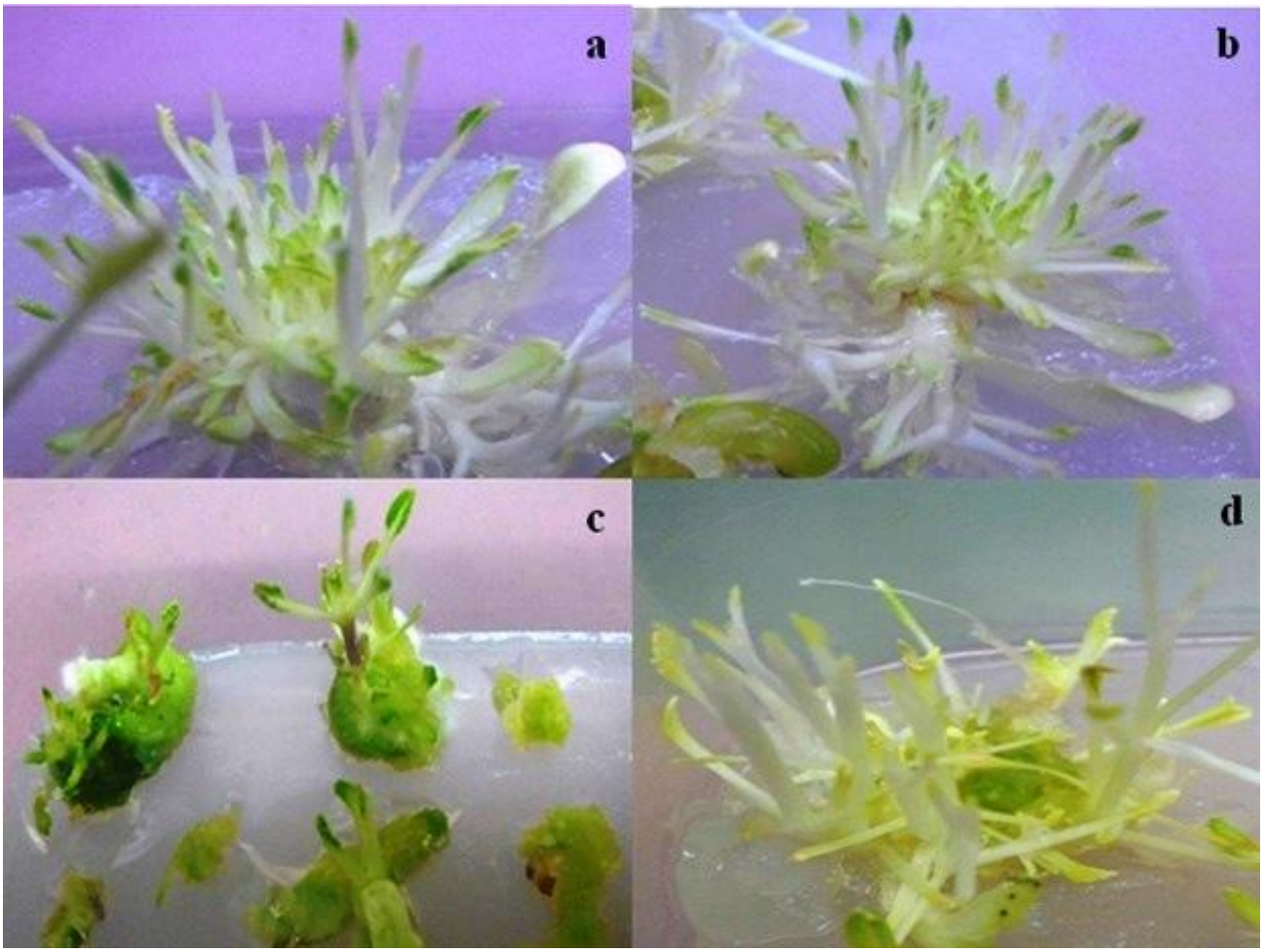


Figure 2. For cotyledon node explant (a) maximum callus formation on CN9 medium, (b) maximum regeneration shoot rate on CN7 medium, (c) maximum number of shoot per explant on CN8 medium, (d) the longest shoot on CN4 medium.

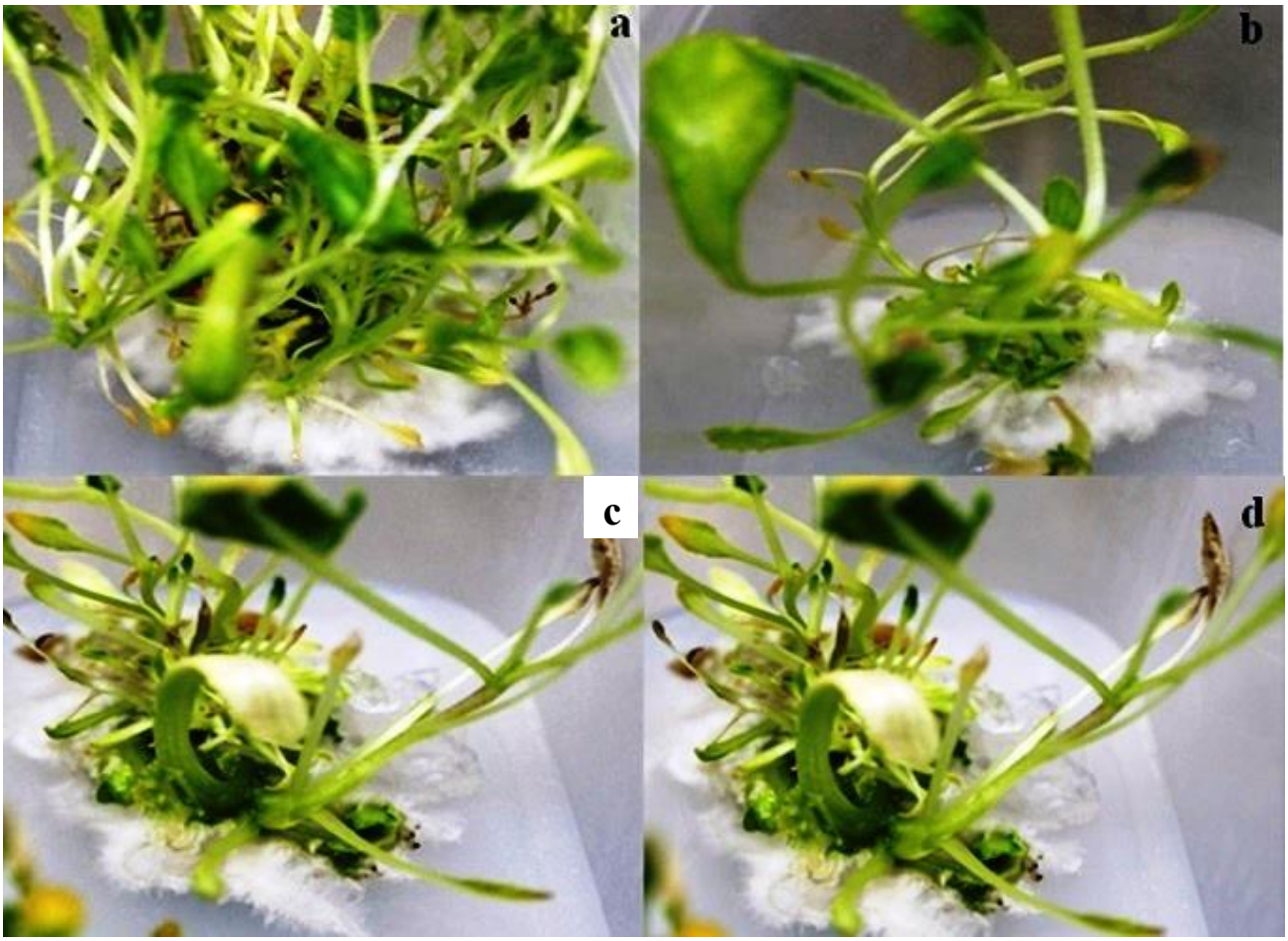


Figure 3. Rooting; (a) MS medium containing 1.00 mg/L NAA, (b) MS medium containing 0.50 mg/L NAA, (c) MS medium containing 2.00 mg/L.

DISCUSSION

Crambe can be used as an oil plant for industrial purposes due to the high erucic acid content of its seeds (Grombacher *et al*., 1993). Micropropagation of crambe and determination of the best necessary hormone concentrations will play a crucial role in the future with regard to plant breeding studies.

BAP and NAA hormone combination is one of the most effective combinations for acquiring shoot and callus formation (Pavalek - Kozlina *et al*., 1999; Tattersall and Millam, 1999; Yang *et al*., 2010; Furmanek and Banas, 2011; Piovan *et al*., 2011; Derelli, *et al*., 2012; Göre, 2014; Özdemir and Türker, 2014; Qi *et al*., 2014; Koç, 2015). Previous studies of crambe regeneration by Furmanek *et al*., 2011; Li *et al*., 2011; Qi *et al*., 2014 have been carried out but there is no study on micropropagation of *Crambe oreintalis* var. *orientalis*.

Qi *et al.* (2014) have studied *Crambe abyssinica* Hochst. ex R.E.Fr. species for genetic transformation. Different BAP and NAA concentrations were attempted for *in vitro* regeneration protocol. Cotyledon and cotyledon nodes were used as explant sources and Microagar and Phytoblend were added in groups for two explant sources. Shoot regeneration percentage was maximum on MS medium containing 0.50 µM NAA + 2.20 µM BAP hormone combination. The authors applied the same cytokinin and auxin hormones and their concentrations were also similar to ours. Therefore the results of that study were in accordance with those of ours.

Leaves and cotyledones of *Crambe abyssinica* cv. Mayer were utilized for primary and secondary callus formation (Furmanek *et al*., 2011). Combinations of cytokinin (BAP and TDZ) and auxin (IAA and NAA) were used in this study. 0.50 mg / dm^{-3} TDZ and 0.50 mg / dm^{-3} BAP + 0.50 mg / dm^{-3} NAA hormone combinations applied to cotyledon explants for primary embryogenic callus formation gave the best result with a rate of 100%. In our study, 0.50 mg/L BAP + 0.50 mg/L NAA hormone combination for cotyledon node and hypocotyl explants formed callus with a frequency of 43.65% and 52.69% respectively. The results are not in agreement with Furmanek *et al*. (2011), who used

different crambe species and hormone combinations than ours.

Carbon source and its amount are among the most essential parameters for micropropagation. Similar results were obtained for *Crambe abyssinica* cv. Galactica by Li *et al*. (2011); who confirmed the use of MS with 30 g / l sucrose for shoot regeneration.

A study carried out previously by Piovan *et al*. (2011) demonstrates that different combinations of BAP and NAA on MS medium are effective for direct organogenesis and somatic embryogenesis. They propagated *Crambe tataria*, an "endangered species" in Italy, in order to protect them under *ex situ* conditions. BAP and NAA hormone combinations yielded the maximum results for both explants (root and leaf) and had a similar rate with that of our study *Camelina sativa* (L.) Crantz that is another species of Brassicaceae family studied for the effect of BAP and NAA hormone combinations on *in vitro* conditions (Tattersall *et al*., 1999). The best shoot regeneration and callus formation was respectively 4.44 µM BAP + 0.54 µM NAA and 0.44 µM BAP + 5.40 µM NAA. In addition, they also noted that BAP and NAA combination was important to root.

Derelli *et al*. (2012) studied the micropropagation of "Clarck" variety of *Linum usitatissimum* L. They found that 1.00 mg/L BAP + 0.02 mg/L NAA was the best hormone combination to regenerate shoots from hypocotyl explants. This study is in agreement with our study about hormone combination and explant source.

Koç (2015) examined the activity of antioxidant enzyme and shoot regeneration *Brassica napus* L. c.v. PR46W31 and the formation of cali and shoots, using BAP and NAA hormone combinations. Results and hormone combination of the present study correspond to this study. The hormone and hormone combination used in cali and shoots formation are in accordance with those of our study.

The propagation of a plant on *in vitro* conditions is important for the protection of genetic sources and plant breeding. An applicable and very important protocol for breeding *Crambe orientalis* var. *orientalis* was developed in our study.

REFERENCES

Derelli, E., P.A. Vaziri, M. Mirzapour and M. Yıldız (2012). The effect of water deficiency originated stress in tissue on shoot regeneration of flax (*Linum usitatissimum* L.) hypocotylex plants. TABAD, 5: 153-156.

Durak, Z.K. (1999). Morphological, anatomical and polinological investigation on some *Crambe* L. species. M.Sc thesis, Deptt. of Biology, Eskişehir Osmangazi University, Eskişehir, Turkey.

Enders, G., and B. Schatz (2013). *Crambe Production*, County Commissions, NDSU and U.S. Department of Agriculture Cooperating, USA.

Furmanek, T., and W. Banas (2011). Embryogenic callus formation by cotyledon and leaf explants of *Crambe abyssinica* seedlings. J. Biotech. Comp. Bio and Bionanotech., 92: 209-213.

Gastaldi, G., G. Capretti, B. Focher and C. Cosentino (1998). Characterization and proprieties of cellulose isolated from the *Crambe abyssinica* Hull. Industrial Crops and Products, 8: 205–218.

Glaser, L.K. (1996). Crambe: An Economic Assessment of the Feasibility of Providing Multiple-Peril Crop Insurance. Economic Research Service for the Risk Management Agency, Federal Crop Insurance Corporation, USA.

Göre, M. (2014). A research to establish effects of explant sources and plant growth regulators on Camelina [*Camelina sativa* (L.) Crantz] tiller and plant induction. M.Sc thesis, Deptt. of Field Crops, Ondokuz Mayıs University, Samsun, Turkey.

Grombacher, A., L.A. Nelson and D.D. Baltenspergern (1993). *Crambe* production. Cooperative Extension, Institute of Agriculture and Natural Resources, University of Nebraska-Lincoln, USA.

Knights, S.E. (2002). *Crambe:* A North Dakota Case Study. Rural Industries Research and Development Corporation, USA.

Koç, Y. (2015). The effects of humic substances on physiological properties, shoot regeneration and antioxidant enzymatic activities *in vitro* culture drapeseed. M.Sc thesis, Deptt. of Biology, Sakarya University, Sakarya, Turkey.

Köybaşı, Ö. (2008). Determination of oil rates and yield for some Crambe species in Çukurova ecology. M.Sc thesis, Deptt. of. Field Crops, Çukurova University, Adana, Turkey.

Lalas, S., O. Gortzi, V. Athanasiadis, E. Dourtoglou and V. Dourtoglou (2012). Full characterisation of *Crambe abyssinica* Hochst. seed oil. J. Am. Oil Chem. Soc., 89: 2253–2258.

Li, X., A. Ahlman, H. Lindgren, and L. Zhu (2011). Highly efficient *in vitro* regeneration of the industrial oil seed crop *Crambe abyssinica.* Industrial Crops and Products, 33: 170–175.

Li, X., E.N. Loo, J. Gruber, J. Fan, R. Guan, M. Frentzen, S. Stymne and L. Zhu (2012). Development of ultra-high erucic acid oil in the industrial oil crop *Crambe abyssinica.* Plant Biotech. J., 10: 862–870.

Murashige, T., and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Phys. Plant., 15: 473–494.

Okhunov, II., Kh. M. Bobakulov, S. F. Aripova, N. D. Abdullaev and V. U. Khuzhaev (2012). Component composition of *Crambe orientalis.* Chem. Nat. Comp., 47: 1018–1019.

Okhunov, II., M.G. Levkovich, N.D. Abdullaev, V.U. Khuzhaev and S.F. Aripova (2011). Alkaloids from *Crambe kotschyana* endemic to Uzbekistan. Chem. Nat. Comp., 47: 487-489.

Özdemir, F.A,, and M. Türker (2014). Effect of different combinations of BAP and NAA *in vitro* propagation of wild safflower (*Carthamus persicus* Wild). YYU J. AgrSci., 24: 30-35.

Pavalek-Kozlina, B., M. Pavlica and M. Vujevic (1999). Micropropagation of *Degenia velebitica* (Deg.) Hay., a Croatian endemic plant species. Phyton (Austria) Special issue: "Plant Physiology", 39: 293-296.

Piovan, A., G. Cassina and R. Filippini (2011). *Crambe tataria*: actions for *ex situ* conservation. Bio. Conserv., 20: 359–371.

Qi, W., I. Tinnenbroek-Capel, J.G. Schaart, B. Huang, J. Cheng, R.G.F. Visser, E.N. Loo and F.A. Krens (2014). Regeneration and transformation of *Crambe abyssinica.* Bio. Med. Cent. Plant Bio., 14: 1-12.

Seyis, F., E. Aydın, and M. Çopur (2013). An alternative oil seed crop: Crambe (*Crambe abyssinica* Hochst. ex R.E. Fries). Anadolu J. Agr. Sci., 28: 108-114.

Stolarski, M., M. Krzyaniak, M. Snieg, M. Christou, and E. Alexopoulou (2013). Production costs and residues evaluation of *Crambe abyssinica* as an energy feedstock. Env. Biotech., 9: 59-64.

Tattersall, A., and S. Millam (1999). Establishment and *in vitro* regeneration studies of the potential oil crop species *Camelina sativa.* Plant Cell Tiss. Org. Cult., 55: 147–149.

Tittonel, E. D. (1995). *Crambe abyssinica* – general outline, AIR3-CT94-2480 *Crambe abyssinica*: A comprehensive programme. Workshop Part 4-Utilization, USA.

Tutuş, A., N. Çömlekcioğlu, S. Karaman and M.H. Alma (2010). Chemical composition and fiber properties of *Crambe orientalis* and *Crambe tataria.* Int. J. Agr. and Bio., 12: 286-290.

Wang, Y.P., J.S. Tang, C.Q. Chu and J. Tian (2000). A preliminary study on the introduction and cultivation of *Crambe abyssinica* in China, an oil plant for industrial uses. Industrial Crops and Products, 12: 47–52.

Yang, X., and B. Al-Duri (2005). Kinetic modeling of liquid-phaseadsorption of reactive dyes on activated carbon. J. Coll. Inter Sci., 287: 25–34.

Yang, J. L., B. Zhao, E.S. Seong, M.J. Kim, W.H. Kang, N.Y. Kim, C.Y. Yu and C.H. Li (2010). Callus induction and high-efficiency plant regeneration via somatic embryogenesis in *Papaver nudicaule* L., an ornamental medicinal plant. Plant Biotech. Rep., 4: 261–267.