CELL BIOLOGY & DEVELOPMENT
Volume 5, Number 1, June 2021 E-ISSN: 2580-4499
Pages: 26-31 DOI: 10.13057/cellbioldev/v050104

Somatic embryogenesis of *Eleusine coracana* on inducted seed explants

HELEN GEBREMEDHEN, TILEYE FEYISSA♥
School of Graduate Studies, Institute of Biotechnology, Addis Ababa University. Addis Ababa, Ethiopia

Manuscript received: 16 March 2021. Revision accepted: 28 May 2021.

Abstract. *Gebremedhen H, Feyissa T. 2021. Somatic embryogenesis of* Eleusine coracana *on inducted seed explants. Cell Biol Dev 5: 26-31.* Finger millet (*Eleusine coracana* (L.) Gaertn.) is one of the most important crops in Africa and India. *E. coracana* contains different nutrients and is a popular food among diabetic patients in different countries. *E. coracana* is an orphan crop in the developing world on which little research has been done. The improvement of this crop is crucial for abiotic and biotic stress tolerance. Tissue culture and transformation techniques are among the approaches to improve this crop. Therefore, the objective of this study is to develop an efficient somatic embryogenesis protocol for the regeneration of different *E. coracana* cultivars. Somatic embryogenesis protocol for regeneration of four cultivars of *E. coracana* is developed. For all cultivars, mature seeds were used for callus induction. After sterilization of the seeds, callus was induced efficiently in different concentrations of 2,4-Dichlorophenoxyacetic acid (2,4-D) in all cultivars. From 95 to100% callus induction were recorded on callus induction medium containing 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L 2,4-D. MS-medium supplemented with 1 mg/L 2,4-D resulted in 45 to 58.3% somaticembryogenesis, and 1 mg/L KN in combination with 0.25 mg/L NAA resulted in the highest meannumber (15.85±0.63) of shoots and 16.74±1.86 roots were recorded from AAUFM-19.

Keywords: Callus induction, kinetin, NAA, shoot regeneration, somatic embryogenesis

INTRODUCTION

The term "millet" is given to some of the different warm-season annual grass crops worldwide harvested as grain for human food or animal feed. The millets are similar to the sorghum forage in their productivity and feed value. The millets have several advantages over sorghum when grown for forage, including no prussic acid potential. In addition to this, they are tolerant soil with high pH conditions. The millets are generally considered negligible crops in other places. Still, India, Africa, and China have great importance in solving the hunger problem of many people in those countries. Compared to other cereal grains, the millets can grow well on less fertile soils and in non-suitable growing conditions (Mark et al. 2012), such as in dry areas, temperate, subtropical, and tropical regions (Baker 1996). In addition, their rapid growth and shorter growing seasons make millets the best for emergency and binary cropping conditions (Marsalis et al., 2012). Millets are Africa's third most important cereal crop after maize and sorghum. They could grow in the harsh semi-arid tropical of Africa, where inadequate rainfall and lack of irrigation make the production of other cereal crops difficult to sustainable. A general impression is that research to improve millets has generally lagged worldwide because they are not grown as food crops in the developed world, and in Africa, they are considered "poor man's crops" (Mywish et al. 1998).

The millets comprise the Panaceae family's five genera (*Panicum, Setaria, Echinochloa, Pennisetum,* and *Eleusine).* Several important cultivated species are: Proso millet (*Panicum miliaceum* L.), Foxtail millet (*Setaria italica* (L.) P.Beauv.), Japanese barnyard millet (*Echinochloa frumentacea* Link), Finger millet (*Eleusine coracana* (L.) Gaertn.), and Kodo millet (*Paspalum scrobiculatum* L.) (Pragya and Rita 2012). Finger-millet (*E. coracana)* gains its name from the head of the plant, which bears some similarities to the splayed hand (Roger 2012). *E. coracana*, native to East Africa, is entwined in the local culture and traditions. However, despite its importance to the livelihoods of millions of small-holder farmers in East Africa, its valuable nutritional and processing properties, the growing demand exceeding supply, and its regional and international trade potential, *E. coracana* have largely been neglected by national and international research organizations and major donors to agricultural research in sub-Saharan Africa. This neglect has contributed to a lack of realization of the potential productivity of *E. coracana.* Increased production, utilization, and trade of *E. coracana* in East Africa are currently limited by several constraints (Mgonja et al. 2005).

Crop damage by insects is minimal, but pests such as birds and *Striga* weed are a constant serious threat to the crop (Esele 1989). The most serious biotic constraint is the blast disease caused by the fungus *Magnaporthe grisea* (T.T.Hebert) M.E.Barr. Blast affects *E. coracana* at all growth stages, particularly causing major losses through neck and Panicle infections (Mgonja et al. 2005). *E. coracana* blast disease is the most devastating, causing over 50% yield loss (Esele 1989). Other constraints to *E. coracana* production include poor incentives and marketing arrangements – low pricing, poor and inaccessible market channels, inaccessibility to credit facilities, and inadequate improved processing and product development facilities at commercial levels (Mgonja et al. 2005).

Newer approaches and technologies for generating new varieties are necessary to meet the growing increase in

cereal demand worldwide, such as creating transgenic plants with desirable traits. Even though millets are economically important, especially in the developing world, little genetic improvement has been made, specifically using wide- or cross-hybridization among closely related species. The incompatibilities due to interspecific hybridization methods are alleviated by directly transferring the desirable traits to millets using the optimum or efficient transformation method. Hence, crossing barriers could be overcome, so the genes from unrelated sources would be introduced asexually into crop plants. On the other hand, monocots in general and cereals in specific were initially difficult to genetically engineer, mainly due to their recalcitrance to in vitro regeneration and their resistance to *Agrobacterium*-mediated infection.

However, efficient transformation protocols have been later established for the major cereals, including rice and maize. Gene transfer to millets would be facilitated once efficient or optimum regeneration has been developed (Sonia and Zerihun 2012). The objective of this study is to develop a somatic embryogenesis protocol for *E. coracana*.

MATERIALS AND METHODS

Plant material

Mature seeds of four cultivars of *E. coracana*, AAUFM-19, AAUFM-14, AAUFM-10, and AAUFM-20, were obtained from the Addis Ababa University Bio-innovative project, which is collected from different localities of the Tigray region, Ethiopia. Out of these, AAUFM-14 is drought susceptible, and the rest three are drought resistant.

Stock solutions and media preparation

Preparation of macronutrient, micronutrient, and vitamin stock solutions

All MS-stock solutions were prepared in different compositions and concentrations of chemicals. For example, for macronutrient stock solution, KNO_3 38 g/L, NH_4NO_3 33 g/L, $CaCl_2.2H_2O$ 8.8 g/L, $MgSO_4$ 7.4 g/L, and KH_2PO_4 3.4 g/L were added together into the one-liter flask and stirred until the contents became dissolved and poured into plastic bottles and stored at -20ºC (Appendix 1).

Preparation of stock growth regulators

Growth regulators 2,4-Dichlorophenoxyacetic acid (2,4-D), α-naphthalene acetic acid (NAA), and kinetin (KN) were used for this study. All growth regulator stock solutions were prepared by weighing and dissolving the powder in double distilled water at the concentration of 1.0 mg/mL. The powder was first dissolved in 3-4 drops of 1M NaOH or HCl and stirred, followed by adding double distilled water and stirring using a magnetic stirrer. Finally, the stock solutions were stored at 4ºC for immediate use.

Culture media preparation

The MS stock solutions (100 mL/l macro, 10 mL/L micro including 10 mL/L iron-EDTA and 10 mL/L vitamins) and different concentrations and combinations of plant growth regulators and 30 g/L sucrose were used to prepare the culture medium in different stages. For callus culture, MS medium supplemented with different concentrations of 2,4-D (1.0, 1.5, 2.0, 2.5, 3.0 mg/L) was prepared. The first 30 g sucrose and MS stock solutions in the above amount were added into1Lvolumetric flask and mixed using a magnetic stirrer, and the pH was adjusted to 5.8 using 1 M HCl and/or 1 M NaOH.

After adding 8 g/l agaroses, the medium was boiled in a microwave oven and autoclaved at 121ºC for 15 minutes. Then 20 mL medium was poured into 90 mm diameter sterile Petri dishes. MS- medium containing 1 mg/L 2,4-D was used for somatic embryogenesis.

MS- medium supplemented with 1 mg/L KN and 0.25 mg/L NAA was used for regeneration from somatic embryos. All the components of MS-medium were added to a volumetric flask and stirred by a magnetic stirrer, then transferred to a beaker and boiled using a microwave oven in order to melt the agar, and 50 mL was poured into Magentas GA-7 culture vessel followed by autoclaving at 121ºC for 15 minutes and kept at 4ºC to be used the next day.

Seed sterilization

About 400 to 500 mature seeds of four-*E. coracana* cultivars were washed under tap water using Ariel powder detergent and rinsed with distilled water. The seeds were then washed in 70% ethanol for 1 min and rinsed three times with sterile distilled water, followed by sterilizing in 20% Clorox containing a drop of Tween-20 for 15 min. Finally, the seeds were rinsed five times with sterile distilled water.

RESULTS AND DISCUSSION

Callus induction

At the first round of callusing, all the cultivars (AAUFM-19, AAUFM-14, AAUFM-10, and AAUFM-20) formed white watery and soft calli in all 2,4-D concentrations (1 mg/L, 1.5 mg/L, 2 mg/L, 2.5 mg/L, and 3 mg/L) after 4 weeks as shown in Figure 1. No callus was induced on a growth regulators-free medium by any cultivar. In further callusing, the calli became a little harder, and some cultivars started to induce somatic embryos, as shown in Figure 2. High percentages of calli were obtained in all cultivars and all 2,4-D concentrations. All the explants (100%) of all cultivars induced callus on medium containing 1 mg/L, 1 mg/L and 1.5 mg/L , 2 mg/L and 2.5 mg/L, and 2.5 mg/L 2.4-D, respectively (Table 1).

Somatic embryogenesis

The eight-week-old calli of all four cultivars resulted in hard, compact, gray, and green mixed-color somatic embryos after two months on a medium containing 1 mg/L 2,4-D (Figure 3).

A maximum percentage (52.5) and (58.3) of the somatic embryo was obtained from calli induced on 2 mg/L 2,4-D in AAUFM-14 and AAUFM-10, respectively. Calli obtained on 1 mg/L 2,4-D resulted in 52.5 % of the somatic

embryo in AAUFM-20, and calli obtained on 3 mg/L 2,4-D produced 56.1% of somatic embryos (Table 2).

Shoot regeneration and rooting

All the cultivars have shown regeneration after four weeks of culturing on an MS medium containing 1 mg/L KN and 0.25 mg/L NAA. Rooting was also successful after eight weeks on the same medium. Multiple shoots were obtained in all cultivars.

Acclimatization

Plantlets of all four cultivars showed 100% survival after acclimatization (Figure 6). During acclimatization, the plants produced additional shoots.

Table 1. The response of callus induction in different concentrations of 2,4- D

Cultivars	2,4-D (mg/L)	Callus induction (%)
AAUFM-19	0.0	0
	1.0	100
	1.5	98.3
	2.0	96.67
	2.5	98.3
	3.0	95
14(100002)	0.0	0
	1.0	100
	1.5	100
	2.0	98.3
	2.5	96.67
	3.0	98.3
AAUFM-10	0.0	0
	1.0	98.3
	1.5	98.3
	2.0	100
	2.5	100
	3.0	96.67
AAUFM-20	0.0	0
	1.0	98.3
	1.5	98.3
	2.0	98.3
	2.5	100
	3.0	98.3

Table 2. Percentage of somatic embryo production from calli on MS medium containing different concentrations of 2,4-D after two months of culture on MS medium supplemented with 1 mg/L 2,4-D

	Cultivar Concentration of 2,4-D in mg/L	No. of calli in culture	No. of calli forming SE	% of calli forming SE
14(100002)	1.0	60	27	45
	1.5	60	30	50
	2.0	59	31	52.5
	2.5	58	29	50
	3.0	59	27	45
AAUFM-10	1.0	59	29	49
	1.5	59	27	45
	2.0	60	35	58.3
	2.5	60	30	50
	3.0	58	30	51.7
AAUFM-20	1.0	59	31	52.5
	1.5	59	29	49
	2.0	59	27	45.7
	2.5	60	30	50
	3	59	27	45.7
AAUFM-19	1.0	60	30	50
	1.5	59	28	47.5
	2.0	58	33	56.9
	2.5	59	28	47.5
	3.0	57	32	56.1

Table 3. Shoot regeneration and rooting in the four cultivars of *E. coracana* in calli resulted from different concentrations of 2,4-D on MS medium containing 1 mg/L KN in combination with 0.25mg/L NAA

	Cultivar Concentration of 2,4-D (mg/L)	Mean No. of shoots/somatic embryo (±SE)	Mean No. of roots/explant (±SE)
14(100004)	1.0	9.70 ± 0.80^{c}	5.89 ± 1.01^{c}
	1.5	9.63 ± 0.68^{c}	7.15 ± 0.88^{c}
	2.0	10.67 ± 0.75^{c}	6.15 ± 1.18^{c}
	2.5	10.04 ± 0.84^{c}	7.41 ± 0.91^{c}
	3.0	10.30 ± 0.78^{c}	6.93 ± 1.19^{c}
AAUFM-10	1.0	13.37 ± 0.74^{b}	11.85 ± 1.18^{b}
	1.5	13.26 ± 0.75^{b}	10.37 ± 1.38^{bc}
	2.0	14.00 ± 0.73^{ab}	11.89 ± 1.26^{b}
	2.5	13.03 ± 0.62^{b}	9.85 ± 1.45^{bc}
	3.0	12.56 ± 0.49^{b}	9.78 ± 1.36^{bc}
AAUFM-20	1.0	13.48 ± 0.59^{b}	13.00 ± 1.68^{ab}
	1.5	13.19 ± 0.59^{b}	11.11 ± 1.48^{bc}
	2.0	12.59 ± 0.62^{bc}	11.37 ± 1.69^{bc}
	2.5	12.00 ± 0.75^{bc}	9.85 ± 1.47^{bc}
	3.0	10.93 ± 0.55^{c}	10.19 ± 1.58^{bc}
AAUFM-19	1.0	14.56 ± 0.72^{ab}	16.74 ± 1.86^{a}
	1.5	15.85 ± 0.63^{a}	12.04 ± 1.59^{b}
	2.0	14.41 ± 0.52^{ab}	14.04 ± 1.45^{ab}
	2.5	14.33 ± 0.59^{ab}	16.59 ± 1.71^{a}
	3.0	14.67 ± 0.50^{ab}	11.15 ± 1.81^{bc}

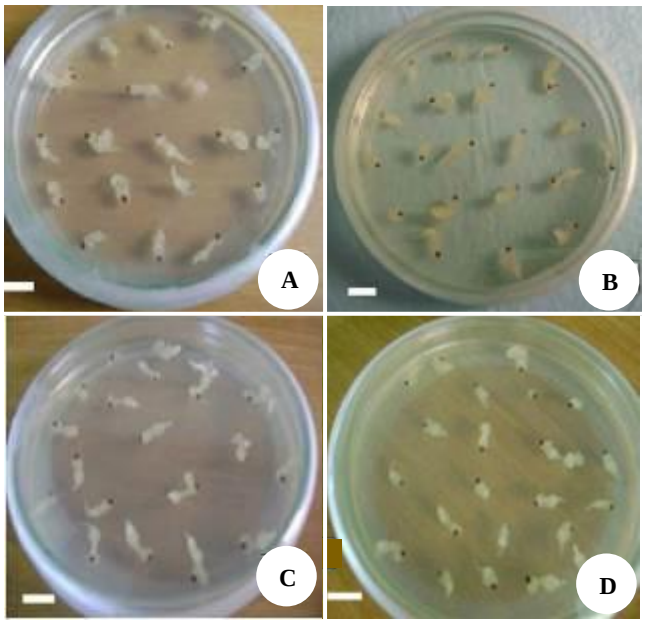

Figure 1. Callus induction after 4 weeks of culture in different concentrations of 2,4-D for the four cultivars (A) AAUFM-10, (B) AAUFM-14, (C) AAUFM-20, and (D) AAUFM-19. Bars represent 2 cm

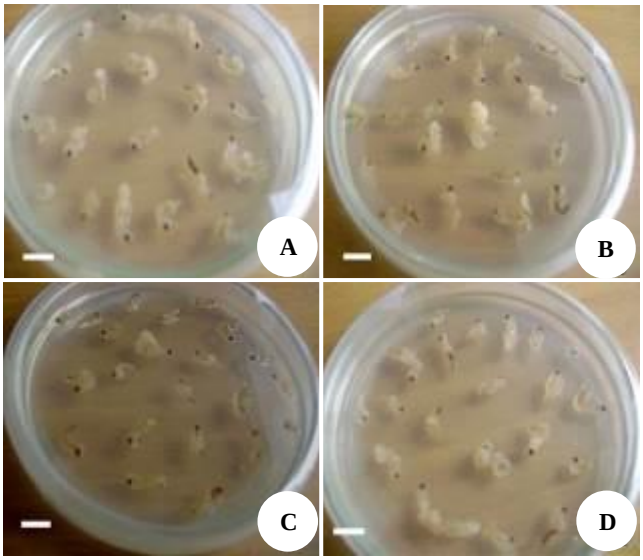

Figure 2. Callus induction from the four cultivars after eight weeks of culture in different concentrations of 2,4-D.(A) AAUFM-10, (B) AAUFM-14, (C) AAUFM-20, and (D) AAUFM-19. Bars represent 2 cm

Figure 3. Somatic embryogenesis after eight weeks of culture on 1 mg/L 2,4-D. Bars represent 2cm

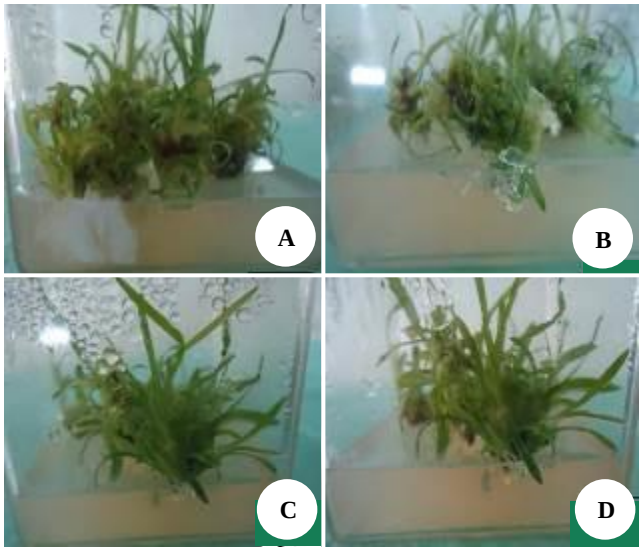

Figure 4. Multiple shoot regeneration from the somatic embryo after four weeks of culture in MS- medium supplemented with 1 mg/L KN in combination with 0.25mg/L NAA. (A) AAUFM-10, (B) AAUFM-14, (C) AAUFM-20 and (D) AAUFM-19

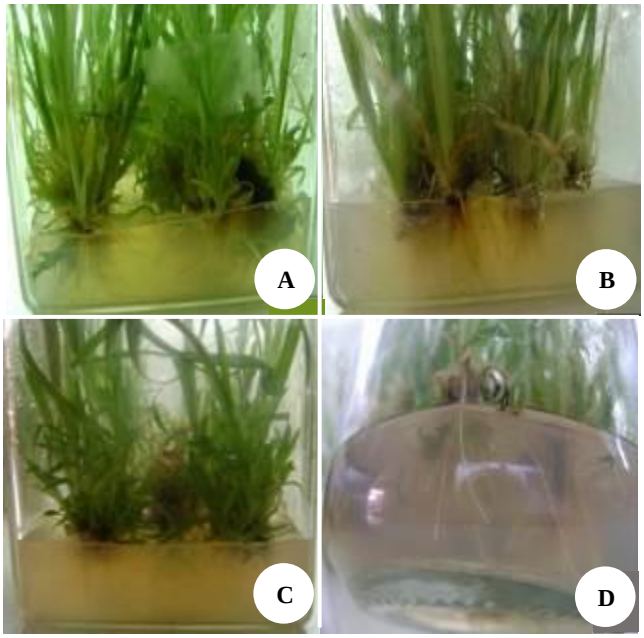

Figure 5. Formation of roots in four extended weeks after regeneration. (A) AAUFM-10, (B)AAUFM-14, (C) AAUFM-20 and (D) AAUFM-19.

Figure 6. Acclimatized plants of the four cultivars of *E. coracana* after 6 weeks

Discussion

Callus induction from seed culture

In this study, five different concentrations of 2,4-D (1.0, 1.5, 2.0, 2.5, and 3.0 mg/L) and growth regulators-free media were used for each cultivar (AAUFM-19), AAUFM-14, AAUFM-10, and AAUFM-20) for callus induction. All cultivars in all concentrations have shown nearly similar results with 95 to 100% callus induction. No callus was induced on growth regulators-free medium from any cultivar. Assefa et al. (2001) reported a similar result on *E.*

coracana on *Eragrostis tef* and *Ediga*. These authors pointed out that 2,4- D was better than NAA for callus induction in these crops. According to George and Eapen (1990), callus induction in cereals and milletsis commonly achieved by 2,4-D. Faiz and Muhammad (2013) also reported a similar result in different rice genotypes with different concentrations of 2,4-D. However, they reported that the number of calli increased with the concentration of 2,4-D, which was not the case in the present study.

Somatic embryogenesis

This study used 1.0 mg/L 2,4-D for somatic embryo induction from callus in all cultivars. Calli derived from different concentrations of 2,4-D has shown a difference in the efficiency of somatic embryo induction. Calli derived from 2.0 mg/L 2,4-D exhibited 58.3% embryo formation in cultivar AAUFM-10, whereas 52. A 5% somatic embryo formation was achieved from both AAUFM-14 and AAUFM-20 cultivars. On the other hand, 56.1% of somatic embryos were produced by calli derived from 1.0 mg/L 2,4-D in cultivar AAUF-19. The above result indicates a slight difference among cultivars within a concentration of 2,4-D in the efficiency of somatic embryogenesis. There was an influence of genotype, media, and explants on the amount of embryogenic callus formation. Jha et al. (2009) reported that callus derived from seed explants were initially loose and watery in nature and became embryogenic after three to four subcultures.

On the contrary, callus induced from immature inflorescences were nodular, hard, and compact and had high renewability. Shoot tip-derived callus was of intermediate quality and required two to three subcultures before becoming embryogenic. They reported that the maximum (80%) embryogenic callus was obtained from immature inflorescence-derived callus in pearl millet (*Pennisetum glaucum* (L.) R.Br.) genotypes. The present study obtained the highest percentage of somatic embryo formation at 1.0 and 2.0 mg/L 2,4- D from seed-derived callus. In many plants, 2,4-D has been widely regarded as effective for somatic embryogenesis (Brown et al. 1995). In addition, embryonic cells are easily formed on media supplemented with 2,4-D for carrot explants (Jiménez and Bangerth 2001, as cited in Mousavizadeh et al. 2010). Also, 2,4-D produced a high frequency of somatic embryos in s*orghum bicolor* (L.) Moench from leaf segments (Sudhakara and Sarada 2006).

Shoot regeneration and rooting

In this step, somatic embryos of the four cultivars derived from different concentrations of 2,4-D were transferred to 1 mg/L 2,4-D for embryogenic callus formation and then transferred to a medium supplemented with a combination of KN and NAA to evaluate their regeneration capacity. Different cultivars resulted in the different mean number of shoots. The highest mean number of shoots (15.85) was obtained from calli derived from 1.5 mg/L 2,4-D in AAUFM-19. The mean number of shoots, 10.67±0.75 and 14.00±0.73, was obtained from calli derived from 2 mg/L 2,4-D in cultivar AAUFM-14 and AAUFM-10, respectively. Calli derived from 1.0 mg/L 2,4-D resulted in a mean number of 13.48±0.59 plantlets in cultivar AAUFM-20. The difference in regenerative capacity between calli resulted from different concentrations of 2,4-D. Somatic embryos of all cultivars were obtained from calli that were induced on different concentrations of 2,4-D induced plantlets in different frequencies in MS-medium supplemented with 1.0 mg/L KN and 0.25 mg/L NAA. Rooting proceeded in a similar medium for a further four weeks, and different cultivars of *E. coracana* exhibited different rooting efficiency. The mean number of roots (7.41±0.91) was obtained from cultivarAAUFM-14 on a medium containing 2.5 mg/L 2,4-D, whereas 11.89±1.26 roots were produced by cultivarAAUFM-10 on 2.0 mg/L 2,4-D. Similarly, cultivars AAUFM-20 and AAUFM-19 produced 13.00±1.68 and 16.74±1.86 mean number of roots on a medium containing 1.0 mg/L and 2.5 mg/L 2,4-D, respectively. These results show a significant difference among calli from different concentrations of 2,4-D in the four-*E. coracana* cultivars. Since the same treatment was used for all cultivars in the rooting medium, this difference could be due to genotype differences. The mean difference was significant at $p>0.05$ among cultivars, but there was no significant difference in the regeneration capacity of calli derived from different concentrations of 2,4-D in a specific cultivar. Genotype has often been considered an important factor in determining the response of in vitro regeneration (Mahmuda et al., 2003). KN and NAA played an important role in giving multiple shoots and rooting efficiency in all cultivars. A similar result was reported by Anjaneyulu et al. (2011) in *E. coracana*. They have reported that KN with a low concentration of NAA resulted in a high mean number of shoots and good rooting. Behzad et al. (2013) also observed a positive effect of KN and NAA on root formation in Matthiola incana.

Acclimatization

All cultivars have shown 100% survival, but there were some morphological differences among the cultivars in terms of the number and appearance of shoots. In cultivar AAUFM-14, most of the plants only increased in height and a slight change in width of their shoots, but there was no increase in several shoots. Cultivars AAUFM-10 and AAUFM-20 exhibited an increase in the number and height of shoots.

In conclusion, somatic embryogenesis and regeneration protocols have been developed through callus culture for four *E. coracana* cultivars, AAUFM-10, AAUFM-14, AAUFM-20, and AAUFM-19. (i) 2,4-D appeared to be effective for callus induction at different concentrations for all cultivars. Therefore, it was used in different concentrations (1-3 mg/L) for all cultivars of *E. coracana* and resulted in 95-100% of callus induction in each cultivar, (ii) For somatic embryogenesis, a medium supplemented with 1 mg/L 2,4-D was found to be the best concentration in the four cultivars of *E. coracana*, (iii) Best shoot regeneration and rooting was obtained on MS-medium supplemented with1 mg/L KN and 0.25 mg/L NAA in all cultivars of *E. coracana*, (iv) There was a significant difference between cultivars. Still, there was no

significant difference in calli derived from different concentrations of 2,4-D in a specific cultivar. Therefore, acclimatization was 100 % successful, and all the plants were healthy in all cultivars.

REFERENCES

Anjaneyulu E, Idress HA, Himalatha S, Bharath SR, Balaji M. 2011. An efficient protocol for callus induction and plant regeneration in finger millet (*Eleusine coracana* L.). World Appl Sci J 12 (7): 919-923.

Assefa K, Tefera H, Merker A, Kefyalew T, Hundera F. 2001. Quantitative trait diversity in tef [Eragrostis tef (Zucc.) Trotter] germplasm from Central and Northern Ethiopia. Genet Resour Crop Evol 48 (1): 53-61. DOI: 10.1023/A:1011245918356.

Baker RD. 1996. Millet production. Guide A-414. At http://www.google.com. p.6.

Behzad K, Afshin AH, Alireza T, Sahar BZ, Davood H, Mohammad HA. 2013. Effect of kinetin (Kn) and naphthalene acetic acid (NAA) on the micropropagation of *Matthiola incana* using shoot tips, and callus induction and root formation on the leaf explants. Afr J Agric Res 8 (30): 4134-4139.

Brown DC, Finstad KI, Watson EM. 1995. Somatic embryogenesis in herbaceous dicots. In: Therope TA (Eds). In vitro Embryogenesis in Plants. Kluwer Academic, Netherlands. DOI: 10.1007/978-94-011-0485-2_10.

Ediga A, Attitalla I, Hemalatha S, Bharath Raj S, Balaji M. 2001. An efficient protocol for callus induction and plant regeneration in finger millet (*Eleusine coracana* L.). World Appl Sci J 12 (7): 919-923.

Esele JP. 1989. Cropping systems, production technology, pests and diseases of finger millet in Uganda. In: Seetharam A, Riley KW, Harinarayana G (Eds). Small Millets in Global Agriculture. Ottawa, Canada.

Faiz AJ, Muhammad SK. 2013. Scutellum-derived callus-based efficient and reproducible regeneration system for elite varieties of indica rice in Pakistan. Intl J Agric Biol 15: 27-33.

George L, Eapen S. 1990. High frequency plant-regeneration through direct shoot development and somatic embryogenesis from immature inflorescence cultures of finger millet (*Eleusine coracana* Gaertn). Euphytica 48 (3): 269-274. DOI: 10.1007/BF00023660.

Jha P, Yadav CB, Anjaiah V, Bhat V. 2009. In vitro plant regeneration through somatic embryogenesis and direct shoot organogenesis in *Pennisetum glaucum* (L.) R. Br. In Vitro Cell Dev Biol Plant 45 (2): 145-154. DOI: 10.1007/s11627-009-9198-6.

Jiménez VM, Bangerth F. 2001. Endogenous hormone levels in explants and in embryogenic and non-embryogenic cultures of carrot. Physiologia Plantarum 111 (3): 389-395. DOI: 10.1034/j.1399-3054.2001.1110317.x.

Mahmuda K, Hazrat A, Nenita VD. 2003. Effect of genotype and culture media on callus formation and plant regeneration from mature seed scutella culture in rice. Plant Tissue Cult 13 (2): 99-107.

Marsalis MA, Lauriault LM, Trostle C. 2012. Millets for Forage and Grain in New Mexico and West Texas (Guide A-417). College of Agricultural, Consumer and Environmental Sciences, New Mexico State University, New Mexico.

Mgonja MA, Lenné JM, Manyasa E, Sreenivasaprasad S. 2005. Creating opportunities for improving production and utilization of finger millet. In: (Mgonja MA, Lenné JM, Manyasa E, Sreenivasaprasad S (Eds). Finger Millet Blast Management in East Africa. ICRISAT, Kenya.

Mousavizadeh SJ, Mashayekhi K, Akbarpour V, Kalati H, Ghasemi Y. 2010. Effect of IAA and 2,4-D on somatic embryogenesis and pigments synthesis of carrot root secondary phloem. Austral J Agric Eng 1 (4): 126-131.

Mywish M, Derek B, Peter P. 1998. Impacts of Food Crop Improvement Research in Africa. Special Program for African Agricultural Research Occasional Papers Series, No.1 Washington DC.

Pragya S, Rita SR. 2012. Finger millet for food and nutritional security. Afr J Food Sci 6 (4): 77-84. DOI: 10.5897/AJFSX10.010.

Roger B. 2012. Finger-Millet: The Contribution of Vernacular Names Towards its Prehistory. Kay Williamson Educational Foundation, Cambridge.

Sonia PW, Zerihun T. 2012. Millet improvement through regeneration and transformation. Biotechnol Mol Biol Rev 7 (2): 48-61. DOI: 10.5897/BMBR12.001.

Sudhakara RP, Sarada M. 2006. Somatic embryogenesis and plantlet regeneration in *sorghum bicolor* (L.) Moench, from leaf segments. J Cell Mol Biol 5: 99-107.