RESEARCH ARTICLE

Md. Jahangir Alam
Rajesh Barua

Authors' address:
Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh.

Correspondence:
Md. Jahangir Alam
Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh.
Tel.: +8801717318542
e-mail: mja.gebsust@yahoo.com

Article info:
Received: 9 April 2014
Accepted: 6 August 2014

In vitro regeneration and antibacterial activity of *Prunus domestica* L.

ABSTRACT

An effective *in vitro* culture system for mature nodal segments of European plum (*Prunus domestica*) was established. Several important aspects of regeneration from nodal explants were studied for adaptation of European plum in Bangladesh. Leaf explants were used for callus induction and ½ MS media supplemented with 1.0 mg/l indole butyric acid (IBA) + 0.5 mg/l naphthalene acetic acid (NAA) induced low efficiency callus. The nodal segments of 1.5 cm were cut from young plants, sterilized and established *in vitro*. The successful cultures were achieved on MS media supplemented with 2-6 mg/l kinetin (KIN); 2, 3, 5 mg/l benzyleaminopurine (BAP) and 4 mg/l KIN combination with 2 mg/l BAP. The highest number of shoot length (1.20 ±0.15 cm), highest number of shoot (3.00±0.35 cm), and highest number of leaf (14.25±1.45) were observed at KIN 4.0 mg/l. The successful multiple shoots were achieved on MS media with 2-6 mg/l KIN, 4 mg/l KIN combination with 2 mg/l BAP. This indicates that, in general, European plum species is very responsive to *in vitro* regeneration from nodal segment. The shoots from micro cutting were rooted in MS media supplemented with 1.0 mg/l IBA and with 0.5 mg/l BAP; ½ MS media supplemented with 0.5 mg/l IBA + 0.5mg/l NAA and 1.0 mg/l IBA + 0.5 mg/l BAP. Regenerated plantlets successfully acclimatized to grow vigorously with no apparent phenotypic aberrations. In addition, in sense of commercial value without growth hormone achieved great result. Furthermore, the antibacterial activity of extracts of European plum was evaluated against the human pathogenic bacteria *Escherichia coli, Klebsiella pneumonia, Protease mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* by agar disc diffusion method. Most of the human pathogens were resistant against commercial disk. But plum fruit extract showed good inhibition zone result (14.25 mm by crude extract against *P. aeruginosa* and 12.4 mm by dry extract against *S. aureus*). The positive results of screening of this plum for antibacterial activity forms primary platform for further phytochemical and pharmacological studies.

Key words: European plum, tissue culture, shoots regeneration, KIN, antibacterial activity

Introduction

European plum (*Prunus domestica*) is an economically important fruit crop and is widely grown across the world. Plum fruit is rated high in antioxidant content with many health benefits like vitamin A, vitamin B1, vitamin B2, vitamin C, niacin, minerals, calcium, potassium, phosphorus, and iron (Boccia et al., 2004).

The first reports of European plum (*Prunus domestica*) tissue culture came in early 1980 (Druart, 1980) produced in plant regeneration from different *Prunus* species. High doses of cytokinin along with auxins is necessary to organogenesis (Nowak & Miczynski, 2002) and the most efficient in respect appears to be BAP and thidiazuron (TDZ) or in combination with NAA (Escalettes & Dosba, 1993). The high efficiency of BAP is described for regeneration in explants of 'Bluefre'

DOI: 10.69085/jbb20151009

RESEARCH ARTICLE

(Cossio & Bassi, 1991). TDZ has a strong cytokinin activity (Nowak & Miczynski, 2002) to produce big masses of callus.

Shoots induced with MS medium 555 µM inositol, 1.2 µM thiamine HCl, 4.1 µM nicotinic acid, 2.4 µM pyridoxine HCl, 2.5 µM indolebutyric acid (IBA), 25 $g.L^{-1}$ sucrose, 7 $g.L^{-1}$ bactoagar (Tian et al., 2007). Shoots are regenerated from immature cotyledons of *Prunus persica* (peach) and from the same area in mature cotyledons of plum and sour cherry on MS (Mante et al., 1989). Rooting efficiency is very low, as described by Padilla et al. (2003) and Tian et al. (2007). Regenerated shoots of plum are cultured in both light and dark condition and shoots rooted (Mante et al., 1989).

The significance of medicinal plants for prevention, treatment and cure of diseases are always recognized. History revealed that plants have been a valuable source of natural products for maintaining human health. Their importance is continuously increasing day by day (Israr et al., 2012). Most of the people now prefer natural therapies to overcome serious side effects of some of the present day medication (Owolabi et al., 2007). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs (Nascimento et al., 2000). Plum fruits contain copious amounts of natural phenolic phytochemicals, such as flavonoids, phenolic acids, anthocyanins, and other phenolics, which may function as effective natural antioxidants in our daily diet (Weinert et al., 1990; Gil et al., 2002; Cevallos-Casals et al., 2006; Vizzotto et al., 2007; Kristl et al. 2011). *Prunus domestica* extracts are known to contain two major anthocyanins: cyanidin 3-*O* glucoside and cyanidin 3-*O* rutinoside (Adje et al., 2008). Antifungal activity of *Prunus domestica* has been detected by (Mahmood et al., 2010).

The goal of the present study is to evaluate relatively simple *in vitro* protocol for European plum, and to continue plant multiplication throughout the year irrespective of the season. Also, to investigate the plum as a medicinal plant against some human pathogenic bacteria.

Materials and Methods

Plant materials

Leafs and nodes were sterilized by simple modified protocol described by Tian et al. (2007) and Zou (2010). Mature and fresh nodal and leaf explants were washed with tap water, jerking with 70% ethanol for 3 and 2 minutes, respectively. These explants were further cleaned with 0.1% mercuric chloride (Clotech) for 4 and 3 minutes chronologically, then added few drops of Tween-20 for 5-3 minutes (Wong, 1986; Hamill et al., 1993), rinsed under double distilled water. Explants were dried on filter papers, cut with stainless blade between every inter-node. And in leaf explants, cut into 2-3 pieces by tetra angle shape.

Shoot regeneration culture

The nodal explants were cultured on the MS media supplemented with different concentration of BAP, KIN alone and 4 mg/l KIN with different combination with BAP (Table 1). MS media without PGR (plant growth regulator) was taken as positive control. And leaf explants incubated with MS media in different concentration of 2,4-D (1 mg/l, 2 mg/l) alone; 2,4-D (2 mg/l) was also used in combinations with KIN (1mg/l, 2 mg/l, 3 mg/l) and in combination with BAP (2.5 mg/l) + NAA (0.5 mg/l) and 2,4-D (0.5 mg/l, 1 mg/l, 1.5 mg/l, 2.0mg/l) in combination with NAA (0.5 mg/l). In addition, ½ MS media with 2,4-D (2 mg/l) in combination with BAP (3 mg/l, 4 mg/l); 2,4-D (0.5 mg/l, 1 mg/l, 1.5 mg/l, 2.0mg/l) in combination with BAP (2.5 mg/l) + NAA (0.5 mg/l); 1.0 mg/l BAP + 0.5 mg/l NAA and 1 mg/l IBA + NAA (0.5 mg/l, 1 mg/l) were used for callus induction from leaf explants. Number of regenerated shoots and callus were recorded after 30 days. The primary shoots from the initial culture were cut and cultured on MS media supplemented with different concentration of growth hormone KIN (2 mg/l, 3 mg/l, 4 mg/l, 5 mg/l, 6 mg/l) and 4 mg/l KIN combined with BAP (2 mg/l). Each of the treatments were replicated five times, leading to a total of 215 explants.

Rooting and acclimatization

The shoots were transferred to half strength MS medium supplemented with 15 g/l sucrose, 5 g/l agar, different concentrations of 0.5 mg/l NAA combinations with (0.5 mg/l, 1 mg/l) IBA separately. Full strength MS medium was taken with 1 mg/l IBA alone and combination with 0.5 mg/l BAP. The rooted shoots were removed from the culture tubes, washed with tap water and transferred to plastic pots with the mixture of garden soil. The plantlets were placed in outside environment.

Plant materials for antibacterial activity

Selected plum fruit parts were washed, grinded with mortar pestle and crude extracts were filtered through Watmman filter paper No.1 and dissolved in (5 mg/ml)

DOI: 10.69085/jbb20151009

distilled water. Extracts were also dried and both were used to test antibacterial activity.

Determination of antibacterial activity assay by disk diffusion method

The fruits extracts (crude and dry) were tested by the disc diffusion method (Pharmacopiea of India, 1996). The test microorganisms were seeded into nutrient agar (NA) media by spread plate method 10 µl (10^6 cells/ml) with the 24 hours cultures of bacteria growth in nutrient broth. After solidification, the filter paper discs (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. *Escherichia coli, Klebsiella pneumonia, Protease mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus* obtained from U.S.D.A. (U.S. Department of Agriculture) Lab, Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh used for antibacterial test. Streptomycin sulphate (10 µg) used as positive control. The antibacterial assay plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm.

Results and Discussion

The selection of the correct plant part is one important criterion for successful *in vitro* culture. Actively growing nodal segment is recommended because of their low rate of contamination and better culture establishment. In plum, this type of segment is lower practice (Zou, 2010). Commonly used plant parts: hypocotyls (Tian et al., 2007), cotyledons (Mante et al., 1989), leaf (Nowak & Miczynski, 2002), shoot tip (Uematsu & Akihama, 1987) were observed. It is well known, the fact that the propagation of woody fruit species and stone fruit species is difficult through tissue cultures and especially the propagation system through meristem (Jakab et al., 2008).

In aspect of callus induction, after 14 days, it was achieved at 1.0 mg/l IBA + 0.5 NAA with ½ MS medium. But another research, 1.0 mg/l IBA + 0.5 mg/l NAA with ½ MS medium successfully induced *in vitro* condition (Nowak & Miczynski, 2002). Nowak & Miczynski (2002) suggested TDZ for big masses callus induction. But in the study, alternative hormone (IBA + NAA) was used to low efficiency callus.

Table 1. *Shoot initiation from nodal explants.*

PGR	Concentration (mg/l)	Day to shoot initiation (mean±SE)	Length of shoot at 4 weeks (cm) (mean±SE)	No. of shoot at 4 weeks (mean±SE)	No. of leaf at 4 weeks (mean±SE)	Regeneration (%)
Without PGR		14.00±0.50	0.48±0.07	1.00±0.00	8.75±1.98	80%
BAP	1.0	-	-	-	-	-
	2.0	10.83±2.61	0.47±0.10	1.00±0.00	3.17±1.01	60%
	3.0	18.40±0.78	0.34±0.04	1.00±0.00	1.80±0.18	50%
	4.0	-	-	-	-	-
	5.0	11.50±0.35	0.25±0.12	1.00±0.00	2.00±0.71	40%
	6.0	-	-	-	-	-
KIN	1.0	-	-	-	-	-
	2.0	9.25±2.68	0.68±0.15	1.25±0.22	12.50±1.68	80%
	3.0	7.00±1.41	1.03±0.12	1.33±0.27	11.33±0.72	60%
	4.0	3.25±0.25	1.20±0.15	3.0±0.35	14.25±1.45	80%
	5.0	7.33±1.19	0.50±0.05	2.00±0.47	4.67±0.98	60%
	6.0	12.5±1.77	0.50±0.00	2.00±0.71	5.00±0.71	40%
KIN+BAP	4.0 + 2.0	17.50±1.77	0.60±0.07	2.00±0.71	7.50±2.47	40%
	4.0 + 3.0	-	-	-	-	-
	4.0 + 4.0	-	-	-	-	-
	4.0 + 5.0	-	-	-	-	-
	4.0 + 6.0	-	-	-	-	-

'-' means no result

DOI: 10.69085/jbb20151009

RESEARCH ARTICLE

In present study, the nodal explants were sterilized by 1.0% mercuric chloride with two drops Tween-20 for 15 min, and finally washed again four times (2 min/time) that have also been used by Zou (2010) and Tian et al. (2007). The total experiments of nodal explants are given in Table 1.

Effect of growth regulator

In tissue culture, PGRs are critical media components in determining the developmental pathway of plant. KIN (2 mg/l, 4 mg/l) obtained highest regeneration frequency (80%) as well as treatment without PGR was also gotten. Highest shoot length (1.20 ± 0.15 cm), highest shoot number (3.00±0.35) and highest leaf number (14.25±1.45) were observed at KIN 4.0 mg/l (Table 1). So, KIN is superior hormone among all hormones that were used. Highest mean number of shoots was observed 3.0 (Table 1) while it was observed 18.7 by Mante et al. (1989). Highest mean number of shoots were observed 1.87 (Table 1) recommended by Petri & Scorza (2009). Highest percentage of regenerating leafs was 14.25 (Table 1) while in another research paper it was observed 64.3 (Petri & Scorza, 2009).

Comparing between KIN and BAP

Highest regeneration frequency, 80% was obtained at KIN 4.0 mg/l, whereas 60% was observed at BAP 2.0 mg/l (Figure 1). Besides, highest shoot length was observed 1.20 in KIN while it observed 0.48 in BAP. Likewise, highest shoot number was observed 3.00 in KIN, 1.00 in BAP and highest leaf number was observed 14.25 in KIN, 3.17 in BAP (Table 1). Among BAP and KIN, highest results of shoot length, shoot number and leaf number were achieved by KIN hormone. KIN produced best result at 4.0 mg/l.

Multiple shoots were observed on MS medium with KIN (2.0 mg/l, 3.0 mg/l, 4.0 mg/l, 5.0 mg/l, and 6.0 mg/l) and 2.0 mg/l BAP in combination with 4.0 mg/l KIN (Figures 2 and 3). Zou (2010) achieved successful multiple shoot on WPM (Woody plant medium) with 0.05-0.1 mg/l IBA, 0.2 mg/l BAP, and 0.3 mg/l KIN and 1.0 g/l casein hydrolysate.

In present study, among all hormone (KIN; 2, 4-D and BAP) KIN is the best hormone, especially at 4.0 mg/l. Different combination of hormones were used by Uematsu & Akihama (1987), Mante et al. (1989), Nowak & Miczynski (2002), Padilla et al. (2003), Tian et al. (2007), Jakab et al. (2008), Petri & Scorza (2009), Zou (2010) for shoot initiation. But in present work, only one concentration KIN used for successfully shoot initiation within 12-14 days. This new system may be effective for other plum varieties.

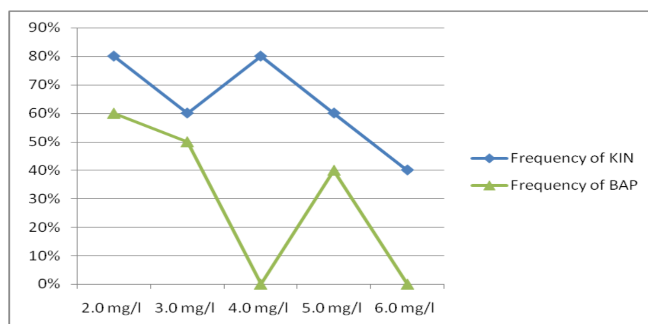

Figure 1. *Comparison between KIN and BAP*

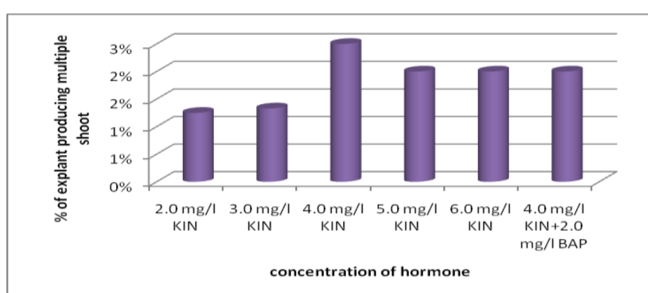

Figure 2. *Effect of multiple shoot induction*

Figure 3. *Multiple shoots*

Rooting and acclimatization

1-1.5 cm *in vitro* regenerated shoots were microcutted and transferred to rooting medium. In this study, we used IBA+BAP, IBA, IBA+NAA that are successfully organized (Table 2). Best root induction showed at 1.0 mg/l IBA concentration. When these shoots were long enough (approximately 0.5–1.0 cm), then they were excised and

DOI: 10.69085/jbb20151009

RESEARCH ARTICLE

transferred to the rooting medium (Tian et al., 2007). According to Padilla et al. (2003) and Tian et al. (2007) addition of higher concentrations of NAA in the medium has been reported to increase rooting. In present study, roots were greatly increased by adding NAA in the ½ MS medium. Highest root number. was appeared 7.0 at 1.0 mg/l IBA. But Petri & Scorza (2009) showed that highest root number appeared 2.3. 37 days were needed from initial culture to the establishment of rooted plants in the outside environment. Mante et al. (1989) established a method of plant regeneration for a time period about 100 days.

Antibacterial pattern

The prevalence of resistant bacteria is significant and deserves more consideration. To overcome this constrain now we are taking shelter to our ancestor's medicinal practice. According to former customs we are taking interest about herbs. Highest percentage of resistance (80%) was observed at ERM against PSA, STRPM against STP and CEPH against STP (Figure 4). Reviewed studies stated that enormous work has done to screen the antibacterial activity of medicinal plants against human pathogen (Jahan et al., 2007; Khan et al., 2007; Rahman & Junaid, 2008; Mishra et al., 2009). The antibacterial efficacy of various plant extracts showed varied level of inhibition against the human pathogenic bacteria (Figure 5B). In present study, crude extract get highly sensitive compared to dry extract.

Table 2. *Root induction of European plum*

Shoot initiation PGR (mg/l)	Root initiation PGR (mg/l), MS	Callus appeared for root (mean) day	Root appeared (mean) day	Root no. (mean)	Root length (mean) cm
Without PGR	0.5 IBA+0.5 NAA, Half	9.00	16.00	4.00	0.3
	1.0 IBA, Full	5.00	10.00	7.00	0.6
	0.5 IBA+0.5 NAA, Half	18.00	09.00	6.00	0.4
2.0 BAP	1.0 IBA+0.5 NAA, Half	18.00	10.00	3.00	0.2
2.0 KIN	1.0 IBA+0.5 BAP, Full	5.00* ± 0.00	11.00	5.00	0.5

'*' means multiple shoot appeared with root

Table 3. *Antimicrobial activities of plum fruit extract against human pathogenic bacterial isolates*

Bacterial isolate	Diameter of inhibition zone (mm) of plum	
	Dry (IZ)	**Fresh** (IZ)
EC	ND	ND
KB	7.6	13.8
PSA	ND	ND
PR	10	14.25
STP	12.4	12.8

IZ= Inhibition zone, ND= Not Detected

DOI: 10.69085/jbb20151009

RESEARCH ARTICLE

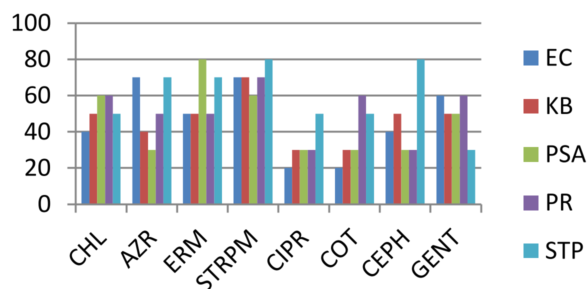

Figure 4. *Percentage of resistance of human clinical bacterial isolates to commercial antibiotics. EC = E. coli; KB = Klebshiella pneumoniae; PSA = Pseudomonas aeruginosa; PR = Proteus mirabilis; STP = Staphylococcas aureus CHL = Chloramphenicol; AZR = Azithromycin; ERM = Erythromycin; STRPM = Streptomycin; COT = Co-trimoxazole; CIPR = Ciprofloxacin; CEPH = Cephradine; GENT = Gentamicin.*

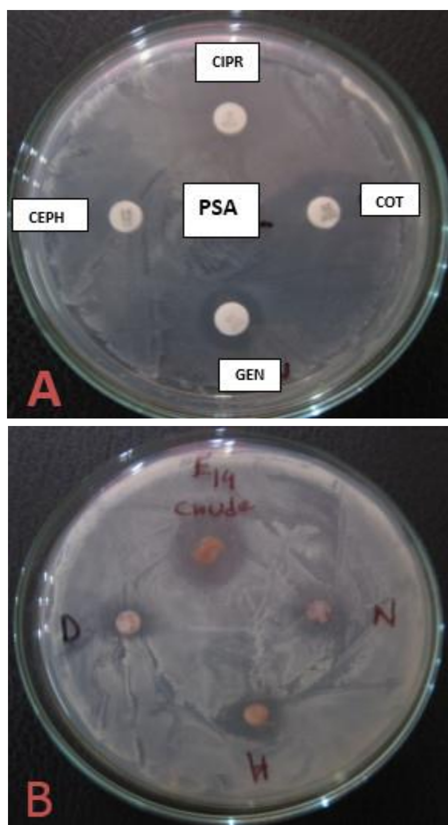

Figure 5. *(A) Different antimicrobial discs. (B) Fresh and dried crude zone against E. coli. 'D' means "dry extract", 'Crude' means crude extract*

Conclusion

In addition, in sense of commercial value without growth hormone achieved great result. In the prospect of Bangladeshi field, it decreases the dependence of import. It could be preferable for some breeding purposes to stimulate this kind of variability during the regeneration process. The obtained results support the use of this plant in medicine. The potential for developing antimicrobials appears rewarding as it leads to the development of new drugs, which is needed today. Further research is necessary to find the active compounds within these plants with their full spectrum of efficacy. However, the present study of *in vitro* antibacterial activity forms primary platform for further phytochemical and pharmacological studies.

References

Adje F, Lozano YF, Meudec E, Lozano P, Adima A, Agbo GN, Gaydou EM. 2008. Anthocyanin characterization of pilot plant water extracts of *Delonix regia* flowers. Molecules, 13: 1238-1245.

Boccia GL, Lucarini M, Lanzi S, Aguzzi A, Cappelloni M. 2004. Nutrients and Antioxidant Molecules in Yellow Plums (Prunus domestica L.) from Conventional and Organic Productions: A Comparative Study. J.Agric. Food Chem., 52(1): 90–94.

Cevallos-Casals BA, Byrne D, Okie WR, Cisneros-Zevallos L. 2006. Selecting new peach and plum genotypes rich in phenolic compounds and enhanced functional properties. Food Chem., 96: 273-280.

Cossio F, Bassi G. 1991. Ricerche sulla organogenesi di alcuni *Prunus*: rigenerazione "in vitro" di germogli di susino (*Prunus domestica* L.) cultivar "Bluefre" [Investigation on organogenesis of some Prunus: "in vitro" shoot regeneration of Prunus domestica L. 'Bluefre"]. Rivista di Frutticoltura, 3: 77-81 [in Italian].

Druart P. 1980. Plant regeneration from root callus of different *Prunus* species. Scientia Horticulture, 12: 339-342.

Escalettes V, Dosba F. 1993. *In vitro* adventitious shoot regeneration from leaves of *Prunus* spp. Plant Science, 90: 201-209.

Gil M, Tomas-Barberan F, Hess-Pierce B, Kader A. 2002. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin A contents of nectarine, peach, and plum cultivars from California. J. Agr. Food Chem., 50: 4976-4982.

Hamill SD, Sharrock SL, Smith MK. 1993. Comparison of decontamination methods used in initiation of banana tissue cultures from field-collected suckers. Plant Cell, Tissue Organ Culture, 33: 343-346.

Israr F, Hassan F, Naqvi BS, Azhar I, Jabeen S, Hasan SMF. 2012. Studies on antibacterial activity of some traditional medicinal plantsused in folk medicine. Pakistan journal of pharmaceutical science, 25(3): 669-674.

DOI: 10.69085/jbb20151009

Jahan T, Begum, ZA, Sultana S. 2007. Effect of neem oil on some pathogenic bacteria. Bangladesh Journal of Pharmacology, 2(2): 71-72.

Jakab IZ, Pamfil D, Clapa D, Fira A. 2008. *In vitro* regeneration and meristem culture of *Prunus domestica* cv. Bulletin UASVM, Horticulture, 65(1): 126-131.

Khan A, Rahman M, Islam S. 2007. Antibacterial, Antifungal and cytotoxic activities of tuberous roots of *Amorphophallus campanulatus*. Turkish Journal of Biology, 31: 167-172.

Kristl J, Slekovec M, Tojnko S, Unuk T. 2011. Extractable antioxidants and non-extractable phenolics in the total antioxidant activity of selected plum cultivars (*Prunus domestica* L.): Evolution during on-tree ripening. Food Chem., 125: 29–34.

Mahmood A, Fatima I, Kosar S, Ahmed R, Malik A. 2010. Structural determination of prunusins A and B, new C-alkylated flavonoids from *Prunus domestica* by 1D and 2D NMR spectroscopy. Magn. Reson. Chem., 48(2): 151-154.

Mante S, Scorza R, Cordts JM. 1989. Plant regeneration from cotyledons of *Prunus persica,Prunus domestica,* and *Prunus cerasu.* Plant Cell, Tissue and Organ Culture, 19: 1-11.

Mishra KD, Maity P, Hansda D, Bandyopadhyay U. 2009. Biological activities of crude extracts and chemical constituents of Bael, *Aegle marmelos* (L). Indian Journal of Experimental biology, 47: 849-861.

Nascimento GGF, Lacatelli J, Freitas PC, Silva GL. 2000. Antibacterial activity of plant extracts and phytoche micals on antibiotic-resistant bacteria. Braz. J. Microbiol., 31(4): 886-891.

Nowak B, Miczynski K. 2002. The course and efficiency of organogenesis on leaf explants of plum (*Prunus domestica* L.) induced by cytokinin. Electronic Journal of Polish Agricultural Universities, Biotechnology, 5(1).

Owolabi J, Omogbai EKI, Obasuyi O. 2007. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigeliaafricana* (Bignoniaceae) stem bark. Afr. J. Biotechnol., 6(14): 882-885.

Padilla IMG, Webb K, Scorza R. 2003. Early antibiotic selection and efficient rooting and acclimatization improve the production of transgenic plum plants (*Prunus Domestica* L.). Plant Cell Report, 22: 38-45.

Petri C, Scorza R. 2010., Factors affecting adventitious regeneration from *in vitro* leaf explants of 'Improved French' plum, the most important dried plum cultivar in the USA, Annals of Applied Biology, 156: 79–89.

Pharmacopiea of India. 1996. The Indian Pharmacopiea, 3rd Edn., Govt. of India, New Delhi, Ministry of Health and Family Welfare.

Rahman SM, Junaid M. 2008. Antimicrobial activity of leaf extracts of *Eupatorium triplinerve* Vehl. against some human pathogenic bacteria and phytopathogenic fungi. Bangladesh Journal of Botany, 37(1): 89-92.

Tian L, Sibbald S, Subramanian J, Svircev A. 2007. Characterization of *Prunus domestica* L. *in vitro* regeneration via hypocotyls. Scientia Horticulturae, 112: 462-466.

Uematsu C, Akihama T. 1987. Effect of 4 PU on the dormant shoot tip culture of peach, nectarine, sweet cherry and plum. Japan Jj. Breed., 37: 283-290.

Vizzotto M, Cisneros-Zevallos L, Byrne DH. 2007. Large variation found in the phytochemical and antioxidant activity of peach and plum germplasm. J. Am. Soc. Hortic. Sci., 132: 334–340.

Weinert I, Solms J, Escher F. 1990. Diffusion of anthocyanins during processing and storage of canned plums. Food Sci Technol-Leb., 23: 396-399.

Wong WC. 1986. *In vitro* propagation of banana (*Musa* sp.) initiation, proliferation and development of shoot tip cultures on defined media. Plant Cell, Tissue Organ Culture, 6: 159-166.

Zou YN. 2010. Micropropagation of Chinese plum (*Prunus salicina* Lindl.) using mature stem segments. Not. Bot. Hort. Agrobot. Cluj, 38(3): 214-218.

DOI: 10.69085/jbb20151009