7.62 in Figure 1) did not increase the fluorescence intensity. This observation might be explained in the following way. Free CTC is membrane-permeable, but not CTC·Ca^{2+} complex. Therefore, free CTC formed at the CTC·Ca^{2+} containing calcium store of a cell would continuously diffuse out of the calcium stores resulting in very little or no CTC that may be available to bind to Ca^{2+} when pH was increased.

We could estimate the pH of two pools of calcium stores with this method. Endosomes and lysosomes have been reported to contain high concentrations of Ca^{2+} (ref. 20) and these are acidic organelles[18,22]. Endosomes have pH between 6.4 and 6.0, and pH of lysosomes is still lower[18,22]. Endoplasmic reticulum, sarcoplasmic reticulum and specialized organelles formed out of endoplasmic reticulum have been extensively reported to contain the receptor-sensitive calcium stores[20], and they are not acidic. Therefore, we might assume that the calcium store with pH 7.2 could correlate with these stores.

Though sensitivity to pH increased with increase in Ca^{2+} concentration at physiological pH values (Figure 3), very high sensitivity to pH may not be advantageous because the sudden decrease in fluorescence with decrease in pH would affect the resolution of the calibration method in cells. But very low sensitivity between 10 and 40 μM Ca^{2+} is also not desirable. Therefore, the 400 μM to 1 mM Ca^{2+} concentration, encountered at the calcium stores[20,21], that showed a moderate sensitivity to pH make the probe suitable for determination of pH of calcium stores in cells.

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Distribution pattern and heavy metal accumulation in lichens of Bangalore city with special reference to Lalbagh garden

Sanjeeva Nayaka*, D. K. Upreti*,‡, Madhav Gadgil¶ and Vivek Pandey†

*Lichenology Lab, and †Stress Physiology Lab, National Botanical Research Institute, Lucknow 226 001, India
¶Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560 012, India and Biodiversity Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur P.O., Bangalore 560 064, India

Occurrence of 30 species of lichens belonging to 19 genera and 15 families in 12 localities of Bangalore city is reported. The Indian Institute of Science (IISc) campus and Lalbagh garden record the maximum number of 24 and 18 species of lichens respectively, which can be directly attributed to the presence of a variety of trees in the area providing diverse substrate for lichen growth. Heavy-metal accumulation in few prominent lichens of some localities is also analysed. Cr and Pb were maximum in *Chrysothrix candelaris* (L.) Laundon, at AMCO Batteries area with 95.29 and 623.95 μg g^{-1} dry wt. respectively. Fe and Cu were maximum in *Bulbothrix isidiza* (Nyl.) Hale and *Pyxine petricola* Nyl. at IISc campus with 22721 and 338.12 μg g^{-1} dry wt. respectively, while *Lecanora perplexa* Brodo at Lalbagh garden has 531.5 μg g^{-1} dry wt. of Zn. The lichen flora of Lalbagh garden is compared to

‡For correspondence. (e-mail: directornbri@satyam.net.in)

an earlier enumeration. It is interesting to note that in the last 18 years lichen flora of the area has changed significantly, as only four species were common between the two studies. The fast pace of urbanization together with air pollution may probably be the reason for the change in lichen flora of this area. The present number, type of lichen and the level of metals accumulated will be a record for conducting future biomonitoring studies in this fastest-growing city of India.

LICHENS are an outstandingly successful group of symbiotic organisms exploiting a wide range of habitats throughout the world[1]. About 20,000 species of lichens are so far known from the world, among which the Indian subcontinent harbours 2450 (12.25%) species[2]. Lichens have long been recognized as sensitive indicators of environmental conditions. In 1859, Grindson attributed the decline of lichens around city centres to air pollution[3]. Lichens show their sensitivity to air pollution in various ways such as decline in diversity, absence of sensitive species, and morphological, anatomical and physiological changes[4,5]. They are also proved to be good accumulators of many elements, particularly heavy metals and radionucleotides[6–8]. Various heavy metals such as Pb, Zn, Ni, Cu, Hg and Cr, considered as toxic for many other living organisms, may be accumulated simultaneously in one lichen specimen, which appears to be unharmed in many cases[9]. Thus several features of lichen such as lack of protective cuticle and stomata, direct dependence on atmosphere for nutrients, longevity, stability, perennial condition of thallus, high degree of sensitivity to changes in substrate pH and pollutants makes them ideal biological monitors[10–12]. Several studies on lichens in relation to air pollution and metal deposition in different regions of the world carried out by a number of workers are available[13–19]. Further, most of the studies are related to the northern hemisphere and generally in countries with humid climate.

In India, a large number of pollution-monitoring studies with higher plants are available[20–22]. However, such studies utilizing lichens have been started recently[23,24]. The purpose of the present study is to provide information regarding lichen flora of Bangalore city together with the accumulation of five heavy metals in some commonly growing lichen species of the area.

Bangalore, popularly known as the 'Garden City' is situated at 12°57′ north latitude and 77°35′ east longitude, with an elevation of 1000 m above mean sea level (Figure 1). The average rainfall is 800 mm, spread over 8 or 9 months. Bangalore is one of the fastest growing cities in India. During the last few decades it has expanded spatially (Figure 2) to accommodate the growing population and increasing economic activity. Bangalore city is currently estimated to have a population of 7 million (Figure 3). Increasing urbanization accompanied by an increase in industrial and commercial activity has resulted in deterioration of air quality in the city. Although emission from industrial establishment, biodegradation and uncontrolled burning of garbage accumulated in dumping yards contribute to urban air pollution, the major cause

Figure 1. Map of Bangalore city showing localities surveyed for lichen collection.

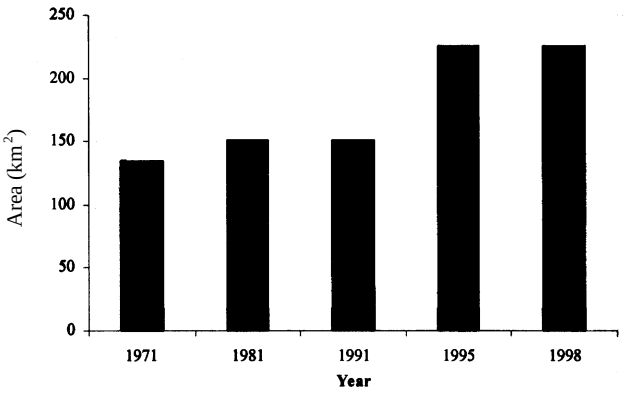

Figure 2. Expansion in area (sq. km) of Bangalore city from 1971 to 1998.

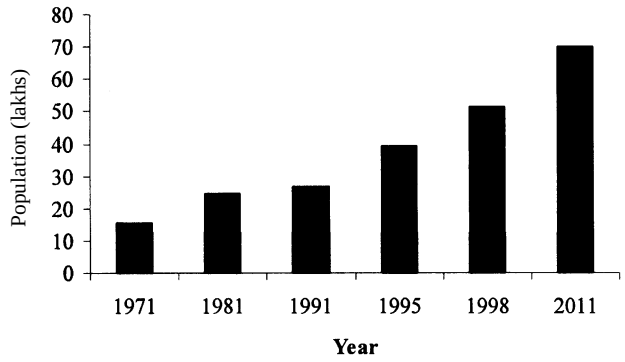

Figure 3. Decadal growth of population (lakhs) of Bangalore city from 1971 and estimation for the year 2011.

for air pollution in Bangalore city is undoubtedly the exhaust emission from vehicular traffic. Inadequate public transport system has led to an increase in the use of personalized vehicles. The vehicular population in Bangalore city during 1997–98 was more than 11 million (Figure 4). Congested traffic, poor road conditions and outdated automotive technology add to the increase in vehicular emissions[25–27].

Lichens from all the available tree species and rocks were collected from 12 localities in Bangalore city (Figure 1) during April 1997. Repeated specimens were often not collected, but only recorded in the field book. The specimens were dried and preserved in the departmental herbarium of Centre for Ecological Sciences, Indian Institute of Science (IISc), Bangalore and a set of voucher specimens were deposited in lichen herbarium of National Botanical Research Institute, Lucknow (LWG).

The specimens were identified by studying their morphology, anatomy and chemistry following recent literature[2,28–33].

Few prominent and common lichen species of the locality were selected for heavy metal analysis. The lichens were carefully removed from the bark and rock using snapper blade and were oven-dried to a constant weight at 80°C. The dried lichen samples (three replicates) were then powdered (0.5 g) for further metal analysis. Chemical analyses of lichen samples were made after extraction with a mixture of concentrated HCl and HNO_3 (3 : 1) and heating to 80°C. Digestion was completed with the addition of a few drops of perchloric acid. The digest was filtered through Whatman filter paper no. 42. The filtrate was diluted to the desired volume with double-distilled water. The total concentrations of Cr, Pb, Fe, Zn and Cu in the filtrate were determined by atomic adsorption spectrometer with a Perkin–Elmer Analyst 300.

The study revealed the occurrence of 30 species of lichens belonging to 20 genera and 15 families out of 400 specimens collected from tree barks and rocks in 12 different localities in and around Bangalore city (Table 1). Among the total species known in the area, 19 are crustose and 11 are foliose forms. Twenty-one species are restricted to growth on trees, five are from rocks while four are common to both the habitats. Among the localities, IISc campus shows maximum number of lichens with 24 species followed by Lalbagh Garden with 18 species, while the K.R. Market area has only two species. The most common species in the city are *Candelaria concolor* (Dicks.) B. Stein, *Chrysothrix candelaris* (L.) Laundon, *Pyxine cocoes* (Swartz.) Nyl. and *P. petricola* Nyl.

The heavy metal analysis studies (Table 2) revealed the maximum accumulation of Cr and Pb in *C. candelaris* (L.) Laundon in AMCO Batteries area with 95.29 and 623.95 $\mu g\ g^{-1}$ dry wt. respectively. Fe and Cu are maximum in *Bulbothrix isidiza* (Nyl.) Hale, and *P. petricola* Nyl. in IISc campus with 22721 and 338.12 $\mu g\ g^{-1}$ dry wt. respectively. The Zn metal shows its maximum concentration in *Lecanora perplexa* Brodo at Lalbagh garden with 531.5 $\mu g\ g^{-1}$ dry wt.

The occurrence of maximum number of lichen species in IISc campus and Lalbagh garden can be directly attributed to the presence of a variety of tree species which provide diverse substrates for lichen growth. The moist and shady condition in both areas also provide suitable habitat for lichens. In other localities of the city, a few avenue trees (*Delonix regia*, *Peltophorum ferrugineum*,

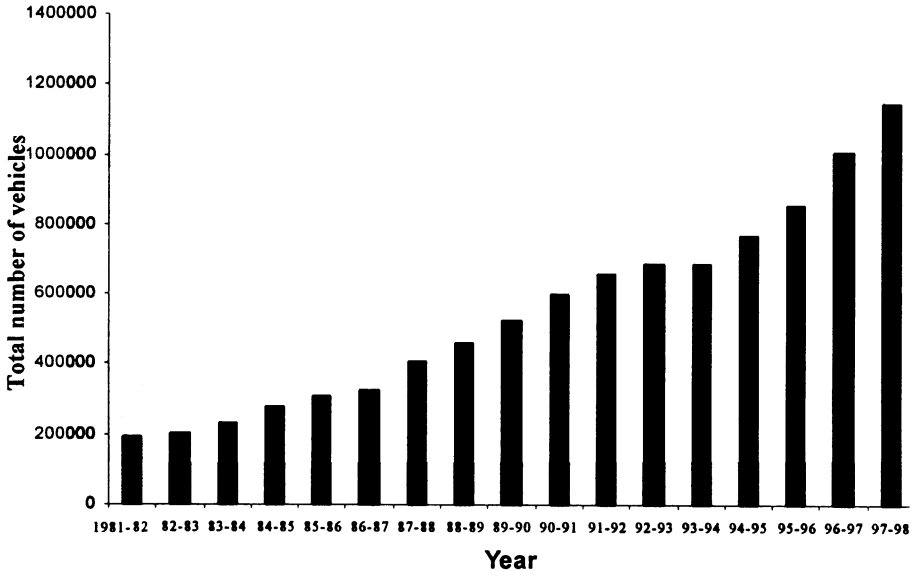

Figure 4. Vehicular population in Bangalore city from 1980–81 to 1997–98.

Samanea saman, *Dolichandrone platycalyx*, *Kigalia pinnata*, *Pongamia pinnata*, *Tabebuia* sp., *Cassia* sp., *Ficus* sp., *Polyalthia* sp.) growing in open dry places do not provide suitable habitats for many lichen species to grow. It is observed that though the city exhibits a variation in lichen diversity in different localities, heavy metal pollution is more or less similar in all the twelve localities. This is because dispersion of the pollutant between the source and receptors is a process which depends upon meteorological conditions and hence is variable. After emission, pollutants disperse both vertically and horizontally in the atmosphere[34].

Table 1. Distribution of lichens in different localities in Bangalore city

Sl. no.	Lichen sp.	GF	Locality 1	2	3	4	5	6	7	8	9	10	11	12	Substratum
	Acarosporaceae														
1	*Thelocarpon palniensis* Awasthi & K. Singh	C	–	–	+	–	–	+	–	–	–	–	–	–	Rock
	Arthopyreniaceae														
2	*Arthopyrenia minor* Reltarris	C	–	–	–	–	–	+	+	–	–	–	–	–	Bark
	Brigantiaceae														
3	*Brigantiaea nigra* Awasthi in Awasthi & Srivastava	C	–	–	–	–	–	–	+	–	–	–	–	–	Bark
	Candelariaceae														
4	*Candelaria concolor* (Dicks.) B. Stein	F	+	+	+	+	+	+	+	–	+	+	+	–	Bark, rock
	Chrysothricaceae														
5	*Chrysothrix candelaris* (L.) Laundon	C	+	–	+	+	–	+	+	+	–	+	+	–	Bark, rock
	Graphidaceae														
6	*Graphis scripta* (L.) Ach.	C	–	–	+	+	–	+	+	–	–	–	–	–	Bark
	Haematommataceae														
7	*Haematomma puniceum* (Sm. ex Ach) Massal	C	–	–	–	–	–	–	+	–	–	–	–	–	Bark
	Hymeneliaceae														
8	*Aspicilia* sp.	C	–	–	–	–	–	–	–	+	+	–	–	–	Rock
	Lecanoraceae														
9	*Lecanora cinereofusca* H. Magn.	C	–	–	–	–	–	–	+	–	–	–	–	–	Bark
10	*L. iseana* Räsänen	C	–	–	–	–	–	–	+	+	–	–	–	–	Bark
11	*L. leprosa* Fée	C	–	–	+	–	–	+	+	+	+	+	+	+	Bark
12	*L. perplexa* Brodo	C	+	–	+	+	–	+	+	–	+	+	+	+	Bark
13	*L. pseudistera*	C	–	–	–	–	–	+	–	–	–	–	–	–	Rock
	Opegraphaceae														
14	*Opegrapha* sp.	C	–	–	+	–	–	+	+	–	–	–	–	+	Bark
	Parmeliaceae														
15	*Bulbothrix isidiza* (Nyl.) Hale	F	–	–	–	–	–	–	+	–	–	–	–	–	Bark
16	*Parmotrema austrosinensis* (Zahlbr.) Hale	F	–	–	–	–	–	+	+	–	–	–	–	–	Bark
17	*P. praesorediosa* (Nyl.) Hale	F	–	–	–	–	–	–	+	–	–	–	–	–	Bark
	Pertusariaceae														
18	*Pertusaria concinna* Erichsen	C	–	–	–	–	–	–	+	–	–	–	–	–	Bark
19	*P. leucosorodes* Nyl.	C	–	–	+	–	–	–	+	–	–	–	–	–	Bark
	Physciaceae														
20	*Buellia stillingiana* Steiner	C	–	–	–	–	–	+	+	–	–	–	–	–	Bark
21	*Dirinaria aegialita* (Afz. in Ach.) Moore	F	–	–	–	–	–	+	+	+	–	+	–	–	Bark
22	*D. consimilis* (Stirton) Awasthi	F	+	–	–	–	–	–	–	–	–	+	+	+	Bark
23	*Heterodermia diademata* (Taylor) Awasthi	F	–	–	–	–	–	+	+	–	–	+	–	+	Bark
24	*H. dissecta* (Kurok.) Awasthi	F	–	–	–	–	–	–	+	–	–	–	–	–	Bark
25	*Physcia tribacia* (Ach.) Nyl.	F	+	–	–	–	–	–	+	+	–	+	+	–	Bark
26	*Pyxine cocoes* (Swartz) Nyl.	F	+	+	+	+	+	+	+	+	–	+	+	+	Bark, rock
27	*P. petricola* Nyl.	F	+	–	+	+	+	+	+	+	+	+	+	+	Bark, rock
	Pyrenulaceae														
28	*Pyrenula nanospora* (A. Singh) Upreti	C	–	–	–	–	–	+	+	–	–	–	–	–	Bark
	Teloschistaceae														
29	*Caloplaca* sp.	C	–	–	–	–	–	+	–	–	–	–	–	–	Rock
30	*Ioplaca pindarensis* (Räsänen) Poelt & Hinter	C	–	–	–	–	–	+	–	+	+	–	–	+	Rock

GF, Growth form/habit; C, Crustose; F, Foliose. Locality: 1, Majestic area; 2, K.R. Market; 3, Jayanagar; 4, Shivajinagar; 5, Malleshwaram; 6, Lalbagh garden, 7, Indian Institute of Science campus; 8, Peenya; 9, Whitefield; 10, Yeshwanthpur; 11, AMCO Batteries; 12, Yenkay.

Table 2. Average heavy metal (μg g^{-1} dry wt) content of lichens in few localities

Locality and lichen species	Cr	Pb	Fe	Zn	Cu
Majestic area					
C. candelaris (L.) Laundon	5.18	ND	7556	95.76	23.72
D. consimilis (Stirton) Awasthi	35.59	149.15	7081	198.14	22.22
Jayanagar					
G. scripta (L.) Ach.	ND	ND	863	384.55	10.06
P. leucosorodes Nyl.	3.04	31.92	570	79.86	5.84
Shivajinagar					
P. cocoes (Swartz) Nyl.	10.31	ND	9795	224.6	19.32
Lalbagh					
D. aegialita (Afz. in Ach.) Moore	ND	ND	7358	122.39	16.06
L. perplexa Brodo	7.96	199.32	265	531.5	7.37
P. austrosinensis (Zahlbr.) Hale	ND	ND	4530	153.92	158.32
P. nanospora (A. Singh) Upreti	36.43	175.9	1506	231.01	18.28
IISc campus					
B. isidiza (Nyl.) Hale	12.99	22.05	22721	102.73	86.02
H. diademata (Taylor) Awasthi (1st sample)	6.62	ND	3020	126.54	1.71
H. diademata (Taylor) Awasthi (2nd sample)	6.82	30.49	4402	160	11.02
P. austrosinensis (Zahlbr.) Hale	ND	ND	586	100.67	20.26
P. praesorediosa (Nyl.) Hale (1st sample)	28.236	164.35	2040	126.85	15.65
P. praesorediosa (Nyl.) Hale (2nd sample)	8.39	233.32	7389	321.21	16.06
P. concinna Erichsen	2.503	ND	55.47	351.68	5.02
P. cocoes (Swartz) Nyl.	9.53	63.63	12056	103.3	16.3
P. petricola Nyl. in Crombie	18.47	101.4	5538	105.38	338.12
Peenya					
C. candelaris (L.) Laundon	13.79	84.69	748	103.82	19.66
D. aegialita (Afz. in Ach.) Moore	34.57	46.4	6887	98.6	8.99
AMCO Batteries					
C. candelaris (L.) Laundon	95.29	623.95	6926	157.496	19.75
L. leprosa Fée	29.92	154	3121	128.15	9.84
P. tribacia (Ach.) Nyl.	ND	191.12	6683	276.47	33.32
Yenkay					
P. petricola Nyl. In Crombie	19	83.33	9202	133.05	115.19

ND, Not detected.

The present lichen flora of Lalbagh garden has been compared to an earlier study carried out during 1980s, when 22 species were reported (Table 3)[35]. However, in the present study 18 species were collected, out of which only four are common to the earlier study. It seems that the remaining 18 species of the former study might have become locally extinct. The change in lichen diversity in the garden is mainly due to changes in the environmental condition during the past 18 years. It is evident from Figures 2 and 3 that there has been a rapid increase in the urbanization of Bangalore city, which has resulted in air pollution and changes in environmental conditions. In a similar study conducted in the Netherlands, decline in lichen diversity and disappearance of sensitive species everywhere in the country within the span of 18 years has been reported[36,37]. In general, crustose and leprose lichens are more tolerant to air pollution followed by foliose and fruticose[38]. In the present study the lichen community of the Lalbagh garden is represented by six foliose species, while in an earlier study 14 foliose species were known from the area and four species are common between the two studies. At present, the garden has dominance of crustose lichens represented by 12 species compared to the earlier eight species. It is interesting to note that none of the crustose lichens are common between the two studies. This indicates the replacement of sensitive species of lichens with tolerant ones in the garden. *C. concolor* (Dicks.) B. Stein, *Parmotrema austrosinensis* (Zahlbr.) Hale, *Pyxine cocoes* (Swartz) Nyl. and *P. petricola* Nyl. are common in both the studies, and seem to be air pollution-tolerant species.

It is clear from the study that air pollution has equally spread throughout the Bangalore city. Lichens along with higher plants act as minor sinks of air pollution by accumulating pollutants at the cost of their life. Increase in air pollution in future will further affect lichen composition

Table 3. Lichen species reported by Awasthi and Upreti from Lalbagh garden during 1980

Lichen species	GF	Sl. no.	Lichen species	GF
Arthonia sp.	C	12	*Lecidea* sp.	F
Buellia inornata (Stirt.) Zahlbr.	C	13	*Ochrolechia subpallescens* Vers.	F
B. isidiza (Nyl.) Hale (Syn. *Parmelia isidiza* Nyl.)	F	14	*Pamelinella wallichiana* (Taylor) Elix and Hale (Syn. *Parmelia wallichiana* Taylor)	
Caloplaca flavorubescens (Huds.) Laundon (Syn. *Caloplaca aurantiaca* (Lightf.) Th. Fr.)	C	15	**Parmotrema austrosinensis* (Zahlbr.) Hale (Syn. *Parmelia austrosinensis* Zahlbr.)	F
**C. concolor* (Dicks.) B. Stein	F	16	*P. tinctorum* (Nyl.) Hale (Syn. *Parmelia tinctorum* Nyl.)	F
Canoparmelia texana (Kurok.) Elix and Hale (Syn. *Parmelia texana* Tuck.)		17	*Physica stellaris* (L.) Nyl.	
Dirinaria applanata (Fée) Awasthi	F	18	*Physciopis adglutinata* (Flörke) Choisy	F
Graphis lineola Ach.	C	19	*Pertusaria* sp.	C
H. dissecta (Kurok.) Awasthi	F	20	**P. cocoes* (Sw.) Nyl. var. *cocoes* and *P. cocoes* var. *prominula* (Stir.) Awasthi	F
H. tremulans (Müll. Arg.) W. Culb.	F	21	**P. petricola* Nyl. var. *petricola* and *P. petricola* var. *pallida* Swinsc. & Krog	F
Lecanora chlarotera Nyl.	C	22	*Pyxine reticulata* (Vain.) Vain.	F

GF, Growth form; C, Crustose; F, Foliose; *, Lichen species common with the present study.

and diversity in this area. The present communication serves as a baseline record regarding the level of heavy metals and number of lichen species for conducting bio-monitoring studies in future.

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In vitro germination of somatic embryos in date palm: Effect of auxin concentration and strength of MS salts

Jameel M. Al-Khayri

Date Palm Research Center, King Faisal University, P.O. Box 400, Al-Hassa 31982, Saudi Arabia

To characterize the germination behaviour of somatic embryos in date palm (*Phoenix dactylifera* L.), embryos derived from callus cultured on hormone-free medium were inoculated on full- or half-strength Murashige and Skoog (MS) medium supplemented with 0, 0.2, 0.4, 0.6, 0.8, and 1 mg l^{-1} naphthaleneacetic acid (NAA) or indole-3-butyric acid (IBA). The embryos either developed complete plantlets, or only shoots or roots. The results indicate a significant interaction between the experimental factors and response. Addition of IBA to the culture medium generally induced higher percentages of complete plantlets compared to NAA at any given concentration. The optimum treatment that maximized the percentage of complete plant formation (86%), consisted of half-strength MS medium containing 0.2 to 0.4 mg l^{-1} IBA. Somatic embryos that formed only shoots ranged from 2 to 26% and were associated with NAA-containing treatments. Generally, NAA enhanced the percentage of embryos that formed only roots, irrespective of medium strength, whereas IBA was inhibitory particularly on half-strength MS medium. Regardless of the germination treatment, 80% of plantlets (192 plants) survived in soil. This study demonstrates the possibility of reducing the length of tissue culture protocols for date palm by merging the germination (shoot formation) and rooting to a one-step procedure.

DATE palm (*Phoenix dactylifera* L.), an economically important commodity, is a monocotyledonous tree widely cultivated in arid regions of the Middle East and North Africa. *In vitro* micropropagation is increasingly becoming an attractive alternative for large-scale propagation of date palm. *In vitro* plant regeneration of date palm occurs through organogenesis and somatic embryogenesis depending on genotype and hormonal manipulations. Explants, including zygotic embryos, shoot tips and lateral buds appear to be most responsive for *in vitro* culture of date palm[1]. Somatic embryogenesis from shoot tip-derived callus has been viewed as the most appealing process for date palm regeneration[2–7]. This method has proved feasibility and agronomic acceptability justifying scale-up of micropropagation for commercial purposes[8]. A typical somatic embryogenesis protocol for date palm involves a series of consecutive stages beginning with callus induction, embryogenic callus multiplication, somatic embryo formation, somatic embryo germination (shoot formation from embryos) and finally rooting. The complexity of the system is magnified by the requirement for different hormonal compositions and lengthy incubation periods associated with each stage, which can be three months. The total incubation translates to periods reaching up to a year or more in some cultivars, to obtain complete plantlets. In addition, another six to 12 months are required in greenhouse nursery before transplanting to the field. Therefore, it is of paramount importance to evaluate the potential of reducing this period so as to enhance the feasibility of commercial micropropagation. This study therefore was conducted to examine the potential of germinating somatic embryos directly on rooting medium. This would allow merging the lateral two stages of culture system, shoot development and rooting.

Previous studies have shown that maturation of somatic embryo, germination, *in vitro* rooting and plant establishment can be influenced by various *in vitro* factors, including solidifying agent, auxin concentration and medium strength in tissue culture systems of numerous plant species[9–13]. Limited studies, however, addressed factors affecting the formation and germination of somatic embryos in date palm such as temporary sucrose starvation[5] and augmenting the culture medium with silver nitrate[14] or biotin[7]. The current study characterized the germination behaviour of date palm somatic embryos and plant establishment in response to salt strength of the medium and various concentrations of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA).

e-mail: jkhayri@kfu.edu.sa