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Article · March 2015

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NBU Journal of Plant Sciences
Vol. 9, No. 1 March 2015 p. 70-77
ISSN No. 09746927

Wetland flora of West Bengal: Lythraceae J. St.-Hilaire

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Abstract
Considering the total area of wide range of wetlands, West Bengal is occupying the fourth position in India. Most of which are known to support very rich plant diversity. The present work recorded a total of 18 species belonging to five genera of Lythraceae J. St.- Hilaire growing in the aquatic or marshland habitat in West Bengal.

Introduction

Lythraceae J. St.-Hilaire is an important family of flowering plants, which is placed under the Myrtales of Malvids of eudicotclade in APG III system of classification (Chase & Reveal, 2009), with about 31 genera and 600 species, widely distributed in tropical regions of the world but relatively less common in temperate regions (Graham *et al*., 2005). C. B. Clarke (1879) in *The Flora of British India* first time gave an account of Lythraceae of British India. There he included 11 genera viz., *Ammannia* Linnaeus (18 spp.), *Hydrolythrum* Hooker *f.* (1 sp.), *Woodfordia* Salisbury (1 sp.), *Pemphis* Forster (1 sp.), *Lawsonia* Linneaus (1 sp.), *Crypteronia* Blume (3 spp.), *Lagerstroemia* Linneaus (12 spp.), *Duabanga* Hamilton (1 sp.), *Sonneratia* Linnaeus *f.* (4 spp.), *Punica* Linnaeus (1 sp.) and *Axinandra* Thwaites (2 spp.). Cook (1995) reported 7 species of *Ammannia* Linneaus, 2 species of *Nesaea* Kunth and 29 species of *Rotala* Linnaeus from the fresh water bodies in India. Wetland areas are very suitable habitat for different annual or biannual herbaceous species of Lythraceae. The fresh and salt water bodies of West Bengal are quite rich in various species of Lythraceae (Chowdhury, 2009; Chowdhury & Das 2010). Nasker (1990) reported 3 species of *Ammannia* and 3 species of *Rotala* from lower Gangetic plains of West Bengal. Prain (1903) described 9 genera viz., *Ammannia* (*Rotala*) (13 spp. and 2 subsp.), *Hydrolythrum* (1 sp.), *Woodfordia* (1 sp.), *Lawsonia* (1 sp.), *Crypteronia* (1 sp), *Lagerostroemia* (4 spp and 1 sp), *Duabanga* (1 sp), *Sonneratia* (2 spp.) and *Punica* (1 sp.) from the pre-independence undivided Bengal. West Bengal is the bottleneck state of eastern India with unique topographic features. It spreads from the Himalayas in the north to the Bay of Bengal in the south along with the central Gangetic plains and *rarh* and small hills of South western Bengal (Purulia and Bankura districts). The state lies between 85° 50' and 89° 50' E longitude and 21°38' and 27°10' N latitude and spreading over an area of 88,752 km^2, shearing the international border with Bangladesh, Nepal and Bhutan. This state is having very wide range of climatic zones viz., tropical, sub-tropical, temperate and sub-alpine along with fresh and salt water wetlands of various sizes and shapes. These wetlands are known to host quite rich with diversified flora and fauna (Chowdhury and Das, 2009, 2010, 2011, 2013, 2014; Biswas *et al*. 2012; Chowdhury *et al*. 2014).

The present study focused on the species of Lythraceae J. St.-Hilaire which are widely growing in different water bodies of West Bengal. Through extensive survey during last 12 years accumulated rich and useful information about these plants from Bengal wetlands. The collected minor taxa of Lythraceae of West Bengal wetlands are enumerated below along with their identification keys, description, distribution and uses.

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ENUMERATION

Key to the Genera:

1a. Aquatic floating herbs; fruits 1- seeded and with 2-4 spines………………………………*Trapa*
1b. Marshy or halophytic rooted herbs or trees; fruits with more than one seed, unarmed…………………………………………….. 2

2a. Halophytic trees; calyx thickly coriaceous.. ………………………………………………*Sonneratia*
2b. Hydrophytic herbs; calyx thin, membranous ……………………………………………………………3
3a. Petals brick-red or absent; capsules open with lateral slit or irregularly at base ………………………………………………………*Nesaea*
3b. Petals pink – purple or absent; capsules open with longitudinal slit or irregulary….. 4
4a. Flowers in axillary cymes ………………………… ……………………………………………*Ammannia*
4b. Flowers solitary or in terminal spikes… ……………………………………………………… *Rotala*

AMMANNIA Linnaeus, Sp. Pl. 1: 119. 1753.

Key to the species:

1a. Styles up to 0.5 mm long…………………………2
1a. Styles 1 – 5 mm long ……………………………3
2a. Lamina and sepals pubescent; sepal appendage distinct……………. *A. verticillata*
2b. Lamina and sepals glabrous; sepal appendage absent…………………… *A. baccifera*
3a. Capsules 2 – 3.6 mm in diameter; sepal lobes erect in capsule……………… *A. multiflora*
3b. Capsules upto 1.5 mm in diameter; sepal lobes reflexed in capsule …………… *A. verticillata*

Ammannia auriculata Willdenow, Enum. P1. Hort. Berol. 1:7,t.7. 1803. Cook, Aqua. Wetl. Pl. Ind. 249.1995.

Hyperhydrate or tenagophyte; erect or decumbent, 10-40 cm tall. Lamina linear-lanceolate, auriculate. Cymes pedunculate. Hypanthium 1.5-3 mm long, vertically 8-10 green ribbed; ribs obscure on fruits. Epicalyx minute. Petals obovate-cuneate or absent; stamens inserted above the middle of the hypanthium; ovary 1-2mm long. Capsule slightly exceeding the hypanthium. Seeds discoid.
Flowers & Fruits: August - November.
Distribution: Tropical and warm temperate regions of World; common throughout the Bengal-plains.

Ammannia baccifera Linnaeus, Sp. Pl. 120. 1753; Clarke in Hooker *f.*, Fl. Brit. Ind. 2: 569. 1879; Prain, Beng. Pl. 2: 500. 1903; Cook, Aqua. Wetl. Pl. Ind. 249.1995.(Plate I: F)

Hyperhydrate or tenagophyte; annual, erect, glabrous herbs. Stem reddish, quadrangular. Leaves opposite decussate; lamina sub- sessile, oblong and narrowly elliptic. Inflorescence axillary, sessile cluster. Flowers pink, bisexual. Capsules globose, irregularly dehiscing, depressed with black seeds.
Flowers & Fruits: July – March.
Distribution: Tropical and warmer regions of Africa, Asia and Australia; abundant throughout the Bengal-plains.
Uses: Leaves used to clear blisters and also given against rheumatism and fever (Misra & Dash, 2002; Pandey *et al.*, 2002).

Ammannia multiflora Roxburgh, Fl. Ind. 1: 447. 1820; Clarke in Hooker *f.*, Fl. Brit. Ind. 570. 1879; Prain, Beng. Pl. 1: 500. 1903; Cook, Aqua.Wetl. Pl. Ind. 250. 1995 (Plate I: B).

Hyperhydrate or tenagophyte; erect branched herbs. Stem quadrangular, hard. Lamina elliptic, sessile. Flowers reddish, axillary, cymes peduncled, compound. Capsules small, globose with persistent style.
Flowers & Fruits: November – February.
Distribution: Tropical and warmer regions of the old World; abundant throughout the plains.

Ammannia verticillata Lamarck, Encycl.[J. Lamarck &*al.*]1(1): 131. 1783; Cook, Aqua.Wetl. Pl. Ind. 251.1995. *Ammannia salicifolia* Heirn in Oliver, Fl. Trop. Afr. 2: 278. 1871. excl. syn.; Clarke in Hooker *f.* Fl. Brit. Ind. 2: 569. 1879; Prain, Beng. Pl.1: 501. 1903.

Hyperhydrate or tenagophyte; erect herbs; stem quadrangular, hard, purple, terete. Leaves opposite decussate; lamina lanceolate, rounded at the base. Inflorescence clusters many flowered, sessile; calyx not covered by calyx teeth. Fruits globose, irregularly dehiscing.
Flowers & Fruits: October – January.
Distribution: Pantropical; abundant throughout the Bengal-plains.

NESAEA Kunth, Nova Genera *et* Sp. Pl. 6 (ed. folio). 1823.

Key to the species:

1a. Lamina base cordate and sub-amplexicauled; petals absent……*N. brevipes*

Plate- I. Lythraceae from wetlands: A. *Rotala rotundifolia* (Buchanan-Hamilton) Koehne B. *Ammannia multiflora* Roxburgh C. *Sonneratia caseolaris* (Linnaeus) Engler D. *Trapaincisa* Siebold & Zuccarini E. *Trapanatans* Linnaeus F. *Ammannia baccifera* Linnaeus G. *Nesaea brevipes* Kiehne

1b. Lamina base attenuate; petals present …………. ………...................................*N. prostrata*

Nesaea brevipes Koehne, Bot. Jahrb .S yst. 3: 326. 1882. *Ammannia cordata* Wight & Arnott, Prodr. 304. 1834, non Hiern; Prain, Beng. Pl. 1: 501. 1903; Cook, Aqua. Wetl. Pl. Ind. 252. 1995 (Plate I: G).

Hyperhydrate or tenagophyte; annual, erect or diffused, glabrous herbs. Leaves opposite, oblong – cordate.Flowers 2 – 6, pedicel short; calyx lobes 4, campanulate, green. Capsules globose with sub hemispheric seeds.

Flowers & Fruits: November – March.

Distribution: Indian sub-continent; abundant throughout the Bengal-plains.

Nesaea prostrata (Buchanan-Hamilton ex Dillwyn) Suresh in D.H. Nicolson, C. R. Suresh & K.S. Manilal, Interpret.Van Rheede's Hort. Malab. 168. 1988; Cook, Aqua. Wetl. Pl. Ind. 252. 1995. *Ammannia prostrata* Buchanan-Hamilton *ex* Dillwyn, Rev. Hortus Malab. 40. 1839; D.J. Mabberley in Taxon 26(5–6): 533. 1977, as *'Ammania'*. 1839.

Hyperhydrate or tenagophyte; annual, erect or decumbent herbs. Lamina linear-lanceolate to oblong. Cyme sessile. Flowers sessile; bracteoles lanceolate to oblong; sepals 4-5; petals 4-5, pink, rarely absent; stamens 4-5. Capsules globose.

Flowers & Fruits: November – March.

Distribution: India, Sri Lanka and Australia; throughout the Bengal-plains; rare.

ROTALA Linnaeus, Mant. Pl. Altera 143. 1771.

Key to the species:

1a. Leaves whorled……………………………………2
1b. Leaves decussate…………………………………3
2a. Racemes terminal; petals present; stamens 4…………………………………… *R. wallichii*
2b. Flowers axillary solitary; petals absent; stamens 1-4 …………………………*R. mexicana*
3a. Flowers in terminal spikes; stigma massive, discoid……………………………*R. rotundifolia*
3b. Flowers in axillary spikes; stigma capitate to punctiform…………………………………………4
4a. Lamina margin translucent to opaque white cartilaginous; capsules 2-valved ...*R. indica*
4b. Lamina margin green, membranous; capsules 3- or 4-valved…………………………5
5a. Stems broadly 4-winged; bracts smaller than foliage leaves; flowers in axillary spikes or sub-sessile on main stem……………………6
5b. Stems terete or 4-angled; bracts like foliage leaves; flowers sessile on main stem…………………………………………*R. rosea*
6a. Epicalyx segments between sepals absent; sepals 4…………………………… *R. cordata*
6b. Epicalyx segments between sepals setiform, about half of corolla tube; sepals 5…………………………………………*R. densiflora*

Rotala cordata Koehne, Bot. Jahrb. Syst. 1: 172. 1880. Cook, Aqua. Wetl. Pl. Ind. 255.1995. *Rotala diversifolia* Koehne, Bot. Jahrb. Syst. 41(2): 77. 1907. *Ammannia cordata* Wight & Arnott, Prodr. Fl. Ind. Orient. 1: 304. 1834. Prain, Beng. Pl. 1: 501. 1903. Clarke in Hooker *f.,* Fl. Brit. Ind. 2: 570.1879.

Hyperhydrate or tenagophyte; annual Herbs. Stem branched, 4-winged. Leaves decussate, narrowly oblong to lanceolate. Bracts lanceolate to oblong. Flowers solitary, subsessile, in bracts of axillary spikes; bracteoles minute, at base of floral tube, scarious. Floral tube 4-merous, broadly campanulate; sepals 4, pink-tinged; petals 4, obovate; stamens 4; ovary globose; style exserted. Capsules globose.

Flowers & Fruits: November – March.

Distribution: NE India, China, Laos, Thailand, Vietnam; throughout the Terai of Darjeeling and Jalpaiguri districts, ascending upto 400 m; less common.

Rotala densiflora (Roth) Koehne, Bot. Jahrd.1: 164. 1880; Datta & Majumdar, Bull. Bot. Soc. Beng. 20 (2): 89. 1966. *Ammannia densiflora* Roth, R. & S. Syst. Veg. 3: 394. 1818. *Ammannia pentandra* Roxburgh, Fl. Ind. 1: 488. 1820; Prain, Beng. Pl. 1: 500. 1903.

Hyperhydrate or tenagophyte; spreading herbs with divarcating floriferous branches, fleshy pink. Leaves elliptic oblong. Flowers small, bracteate, bracteoles scarious, axillary, solitary; epicalyx segments present between sepals, setiform; petals 5, bright pink or white, equal to or surpassing sepals, persistent. Stamens 5. Capsules 3 – 4 valved. Seeds black.

Flowers & Fruits: August – February.

Distribution: Northern parts of India to Australia; common throughout the Bengal-plains.

Rotala indica (Willdenow) Koehne, Bot. Jahrb. Syst. 1: 172. 1880. Cook, Aqua. Wetl. Pl. Ind. 257.1995. *Peplisindica* Willdenow, Sp. Pl. 2: 244. 1799.

Hyperhydrate or tenagophyte; ascending or erect, annual Herbs. Leaves decussate, lamina obovate-elliptic or obovate-oblong, margin translucent to opaque. Bracts leafy or distinctly smaller on axillary spikes.Flowers in axillary spikes or sessile in bracts on main stem; bracteoles linear. Floral tube 4-merous; sepals 4, lanceolate-deltoid; petals 4, pink; stamens 4; ovary ellipsoid; style as long as ovary.Capsules ellipsoid.

Flowers & Fruits: September – April.
Distribution: S & SE Asia; introduced to Africa, Europe and N America; common throughout the Bengal-plains.

Rotala mexicana Chamisso & Schlechtendal, Linnaea 5: 567. 1830. Grierson & Long, Fl. Bhut. 2 (1): 274. 1991. *Ammannia pygmaea* Kurz, J. Bot. 5: 376. 1867; Prain, Beng. Pl. 1: 500. 1903.

Hyperhydrate or tenagophyte; annual, floating, erect or ascending, minute herbs. Leaves whorled, aerial leaves narrowly lanceolate to broadly. Flowers sessile, solitary, axillary; bracteoles linear; petals absent; stamens (1 or) 2 or 3 (or 4), included; ovary subglobose. Fruits capsule.

Flowers & Fruits: September – January.
Distribution: Tropical and warmer regions of world except Pacific Islands, NE Africa, Arabia; throughout the plains North Bengal; rare.

Rotala rosea (Poiret) C.D.K. Cook in Boissiera 29: 86. 1979; Panda & Das, Fl. Sambalp. 150. 2004. *Ammannia rosea* Poiret in Lamarck, Encycl. Meth. Bot. (Suppl. 1) 329. 1810. *A. pentandra* Roxburgh, Fl. Ind. (ed. 1) 1: 448. 1820; Clarke in Hooker *f.*, Fl. Brit. Ind. 2: 568. 1879; Prain, Beng. Pl. 1: 500. 1903.

Hyperhydrate or tenagophyte; annual, terrestrial or amphibious, ascending herbs. Leaves opposite decussate; lamina linear – lanceolate to lanceolate – oblong. Flowers solitary, sessile, axillary; bracteoles linear. Floral tube campanulate; epicalyx segments setiform, equaling sepals; petals 5; stamens 5; anthers reaching margin of floral tube; ovary globose. Capsules globose.

Flowers & Fruits: August – September.
Distribution: S & SE Asia; common throughout the Bengal-plains.

Rotala rotundifolia (Buchanan-Hamilton) Koehne in Bot. Jahrb.1: 175.1881; Bora & Kumar, Flor. Div. Ass. 158. 2003. *Ammannia rotundifolia* Buchanan–Hamilton in Don Prodr. 220. 1825; Clarke in Hooker *f.*, Fl. Brit. Ind. 2: 566. 1828; Prain, Beng. Pl. 1: 500. 1903.(Plate I: A)

Hyperhydrate or tenagophyte; extensively creeping and rooting herbs with red stem. Lamina sessile, orbicular or broadly elliptic – rounded. Flowers pinkish sessile, closely packed in terminal simple or panicle spikes; calyx tube campanulate, sepals 4; petals 4, pink; stamens 4; ovary pyriform to globose. Capsules 4-valved ellipsoid; seeds elliptic peltate.

Flowers & Fruits: November – April.
Distribution: S & SE Asia; abundant throughout the Bengal-plains.

Rotala wallichii (Hooker *f.*) Koehne, Bot. Jahrb. Syst. 1: 154. 1880. Cook, Aqua. Wetl. Pl. Ind. 262.1995. *Hydrolythrum wallichii* Hooker *f.* in Bentham & Hooker*f.*, Gen. Pl. 1: 777. 1867.

Hyperhydrate or tenagophyte; emergent, perennial, Herbs. Leaves whorled; aerial leaves 3-12 whorled, linear to oblong, submerged leaves filiform. Bracts much reduced in inflorescence, oblong or ovate. Flowers 5-8-whorled per node, shortly pedicellate in a bracteate raceme; bracteoles short. Floral tube 4-merous, campanulate; sepals 4; epicalyx absent; petals 4, light red or pink, orbicular; stamens 4; ovary globose; style included. Capsules globose,

Flowers & Fruits: September – April.
Distribution: S & SE Asia; common throughout the Bengal-plains.

Sonneratia Linnaeus *f.*, Suppl. Pl. 38, 252.1782; *nom. cons.*

Key to the species:

1a. Lamina elliptic-lanceolate; stigma peltate………………………………….*S. apetala*

1b. Lamina ovate-oblong; stigma capitate…………………………………………………………………2
2a. Corolla absent; ovary 6-celled…………………………………………………………………*S. griffithii*
2b. Corolla red; ovary 16-20 celled…………………………………………………………*S. caseolaris*

Sonneratia apetala Buchanan-Hamilton, Syems. Embassy Ave. 3: 477. 1800; Prain, Beng. Pl. 1: 505. 1903; Naskar, Pl. Wealth Ganga Delta 1: 351. 1993.'***Tak Keora'***

Intertidal; evergreen tree with erect pneumatophores. Leaves simple, opposite; lamina elliptic, obovate. Inflorescence axillary, 3-flowered cyme. Flowers white; calyx 4 lobed, reflex; petals absent, stamens numerous, filament bent inwards in bud; ovary 2-20 celled, stigma large. Fruit a berry.

Flowers & Fruits: December – July.

Distribution: Native to Bangladesh, India, Myanmar, Sri Lanka, China; inter-tidal river flats of mangrove swamps, muddy flats of Sundarbans; common.

Sonneratia caseolaris (Linnaeus) Engler, Engler & Prantle, Nachtr. 261. 1897; Naskar, Pl. Wealth Ganga Delta 1: 351. 1993. *Rhizophora caseolaris* Linnaeus, Herb. Amboin. (Linnaeus) 13. 1754. '***Chakkeora'*** (Plate I: C)

Intertidal; evergreen tree, glabrous throughout. Pneumatophore present. Leaves opposite; lamina elliptical, oblong or ovate, entire, leathery. Flowers 1-3 at end of drooping twigs malodorous, nocturnal; hypanthium with 6-8 calyx lobes; petals dark or blood-red; stamens numerous, with threadlike filaments; pistil with 16-21 celled; style long, stout. Fruit berry.

Flowers & Fruits: March – October.

Distribution: SE Asia, Philippines, N Australia; common in inter-tidal river flats and inner estuary of mangrove swamps of Sundarbans.

Sonneratia griffithi Kurz, J. Asiat. Soc. Bengal 40 (2): 56. 1871; Naskar, Pl. Wealth Ganga Delta 1: 351. 1993. *Sonneratia acida* Linnaeus; Prain, Beng. Pl. 1: 505. 1903.'***Ora'***

Intertidal; evergreen tree with numerous pneumatophores. Leaves simple, opposite decussate, extipulare; lamina obovate. Inflorescence solitary cyme. Flowers pedicellate; sepals 6 – 8; petals absent; stamens numerous, free; carpels 6, style 1. Fruits berry.

Flowers & Fruits: April – October.

Distribution: Africa, Indo-Malaysia, Australia; common in inter-tidal river flats and outer estuary of mangrove swamps of Sundarbans.

Trapa Linnaeus, Sp. Pl. 1: 120. 1753.

Key to the species

1a. Lamina much villose beneath; margin incised; fruits with 2 soft spines……………………………………*T. natans*
1b. Lamina slightly villose beneath; margin not incised; fruit with 4 stiff spines…….*T. incisa*

Trapa incisa Siebold & Zuccarini, Abh.Math.-Phys. Cl. Königl. Bayer. Akad. Wiss. 4(2): 134. 1845. *Trapa natans* var.*incisa* Makino, Bot. Mag. (Tokyo) 1: 105. 1887 – 1892. *Trapa bispinosa* var. *incisa* (Siebold & Zuccarini) Franchet & Savatier, Nakai, Fl. Kor. 2: 490. 1911. Clarke in Hooker *f*., Fl. Brit. Ind. 2: 590. 1879; Prain, Beng. Pl. 1: 508. 1903. (Plate I: D)

Epihydrate; floating herbs. Floating lamina in rosettes, rhombic – triangular, glabrous or sparsely pubescent on veins, margin coarsely and sharply incised-dentate distally. Petals pink to pale purplish or white. Fruit narrowly rhombic, 4-horned, surface variously ribbed to smooth, crest absent; horns conic.

Flowers & Fruits: May – November.

Distribution: S & SE Asia, China, Japan, Korea, Laos, Malaysia and Vietnam; abundant in stagnant water bodies throughout the Bengal-plains.

Uses: Fleshy larger cotyledon is edible.

Trapa natans Linnaeus, Sp. Pl. 1: 120. 1753. *Trapa natans* Linnaeus var. *bispinosa* (Roxburgh) Makino in Iinuma, Sumoku-Dzusetd "ed. 3", 1: 137.1907; Prain, Beng. Pl.1: 508. 1903. *Trapa bispinosa* Roxburgh, Pl. Cor. 3: t. 234. 1815; Clarke in Hooker *f*., Fl. Brit. Ind. 2: 590. 1879. '***Singara Phal'*** or ***'Pani Phal'*** (Plate I: E)

Epihydrate; floating herbs. Floating leaves in rosettes, rhomboid, crowded in the upper part of stem; submerged ones dissected. Flowers solitary, axillary; calyx lanceolate, acute; corolla white; pubescent; stamens 4. Nuts 3-angled with 2 spiny horns.

Flowers & Fruits: September – January.

Distribution: Tropical parts of Asia; throughout the Bengal plains; commonly cultivated and marketed in large amount.

Uses: Fleshy larger cotyledon is edible.

Discussion

Lythraceae is a family of both terrestrial and wetland elements. 18 species of wetland plants recorded from West Bengal during the present survey and are representing five genera except three tree species of *Sonneratia* that are growing in salt water marshes of Sunderban mangroves, all others are small herbs growing in various depths, mostly as emerge plants near the periphery of water bodies. Majority of the recorded species are widely distributed in the tropical wetlands of the state except *Nesaea prostrata* and *Rotala mexicana*, which are quite rare in distribution and are only recorded from few wetlands of South and North Bengal respectively. Species of *Rotala* and *Neasea* are decumbent fleshy herbs growing at the peripheral marshy zone of different wetlands forming a dense mat along with several species of grasses, sedges and sometimes with isolated *Ammannia* sp.

The occurrence of these species appears to be normal but, considering broadly, the abundant use of herbicides in fields of wetland crops greatly affecting their population in such habitat. Most part of the population of *Sonneratia* spp. is growing near the estuaries but legal and illegal clearing activities are, certainly, affecting its population.

Like any other hydrophyte and/or wetland plants these are also prone to be affected due to different changed conditions in the habitat. If the wetlands are conserved properly then, automatically, most of these species will continue to survive in their natural habitat.

Acknowledgements

The first and second authors are grateful to CSIR & RGNF-UGC for their financial assistance during different phases of the study period. Director, Botanical Survey of India is also acknowledged for permitting the authors to work at CAL.

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Importance of diplazium esculentum (Retz.) SW. (Athyriaceae) on the lives of local ethnic communities in terai and duars of West Bengal -A report

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Plant Archives Vol. 18 No. 1, 2018 pp. 439-442 ***ISSN 0972-5210***

IMPORTANCE OF *DIPLAZIUM ESCULENTUM* (RETZ.) SW. (ATHYRIACEAE) ON THE LIVES OF LOCAL ETHNIC COMMUNITIES IN TERAI AND DUARS OF WEST BENGAL -A REPORT

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Abstract

Diplazium esculentum (Retz.) Sw. or 'Dheki Shak' is used as a nutritive leafy vegetable by the local communities of Terai and Duars parts of West Bengal. From our study and previous literatures it was found of having very important ethnobotanical value. The people of lower socio-economic communities rely mainly upon the collection and selling of this plant during the summer and monsoon season in the study area. The step wise photographs from field to market are represented here along with the ethnobotanical uses by different communities across India.

Key words: *Diplazium esculentum*, Terai and Duars, vegetable, ethnic Communities.

Introduction

There are many naturally growing plant species which are eaten by the local people and even marketed locally but are never cultivated. These are referred as Wild Edible Plants (WEP) (Beluhan *et al*., 2010). These plants are often found in abundance and the people of different cultures and tribes collect these as source of nutrition, medicine etc. Several investigations suggest that sometimes these plants possess more nutritional values than the conventional crops (Grivetti *et al*., 2000). At the same time these do not carry the adverse effects of fertilizers and pesticides used rampantly in our crop fields.

The northern part of West Bengal, lying just at the feet of the Eastern Himalaya is known as Terai and Duars and forms a part of the Himalaya Biodiversity Hotspot which is quite rich in floral diversity (Das *et al*., 2010; Rai and Das, 2008; Shukla *et al*., 2013; Chowdhury, 2015). Due to the prevalence of highly moist and warm climatic conditions for major part of the year, wide variety of plants, including fern and fern allies are growing in abundance in this area. One of the most common fern *Diplazium esculentum* (Retz.) Sw. (commonly called vegetable fern) of family Athyriaceae is abundant in open moist herb land vegetation and the partially open young and circinately coiled fronds of this plant are regularly consumed by local people as a nutritive leafy vegetable. It is known as 'Dhekishak' by Bengalee (Sen and Ghosh, 2011; Panda, 2015), 'Paloi' in Hindi (Panda, 2015), 'Dhekia' by Assamese and 'Okang' by Manipuris (Kutum, *et al.* 2011), 'Sikiomamoidu' or 'Maikhando' by Tripuris (Shil and Choudhury, 2009) etc. Some local people from the lower socio-economic communities collect it in large quantities from the wild regularly and marketed locally and sometime sent to distant markets like in Kolkata. So, this naturally growing fern is a regular source of earning livelihood for these poor local and ethnic peoples except during winter when this species shows very limited growth. The Table 1 presents the uses of this plant as recorded by different workers including Namsa *et al*. (2011), Kagyung *et al*. (2010) and Pegu *et al*. (2013).

The present study aims to represent the detailed ethnobotanical importance of *Diplazium esculentum* (Retz.) Sw. (Athyriaceae) and its local marketability.

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Materials & Methods

During the period of March–July, 2017, a survey was conducted in the Terai and Duars parts of North Bengal. Entire data was collected from the local people who are engaged in its harvest and marketing through discussion using a structured questionnaire. Some collectors were selected from different regions of North Bengal and thoroughly interviewed. The questionnaire was prepared to know all the steps from the collection to marketing and the total process was photographed following the modified method of Chowdhury (2012).

Result & discussion

Instead of all hazards, the harvestors/ gatherers go out routinely to collect this wild vegetable from thick vegetation, mainly the moist herblands, during early summer to late monsoon as this is the most active growth season for the species. They generally collect about 7-10 kg plants every day. After this season, the amount of harvest and the quality drop drastically. After collection they find out one open space, take out the entire harvest generally on a spreading polythene sheet or on matted grasses, clean the materials from the mixed-up twigs of other plants, snails, leeches etc. and made into small 'mutha' (*i.e.* bundles). For binding, they generally use paddy-straw, dry leaf-sheaths of banana plants or grasses. Then the bundles are taken to neighborhood market or 'Haat' or directly to consumers. Sometimes, they directly sell it to passer-by just sitting on the roadside, again, sometimes there are middle-men or a second person to take the material from the collector to the market. The cost varies from 2 to 4 bundles for Rs. 10 in different markets within the study area. During the collection of *D. esculentum* fronds, they also collect some other edible plant parts like the flower buds of *Colocasia esculentum* and sell these together (PLATE I).

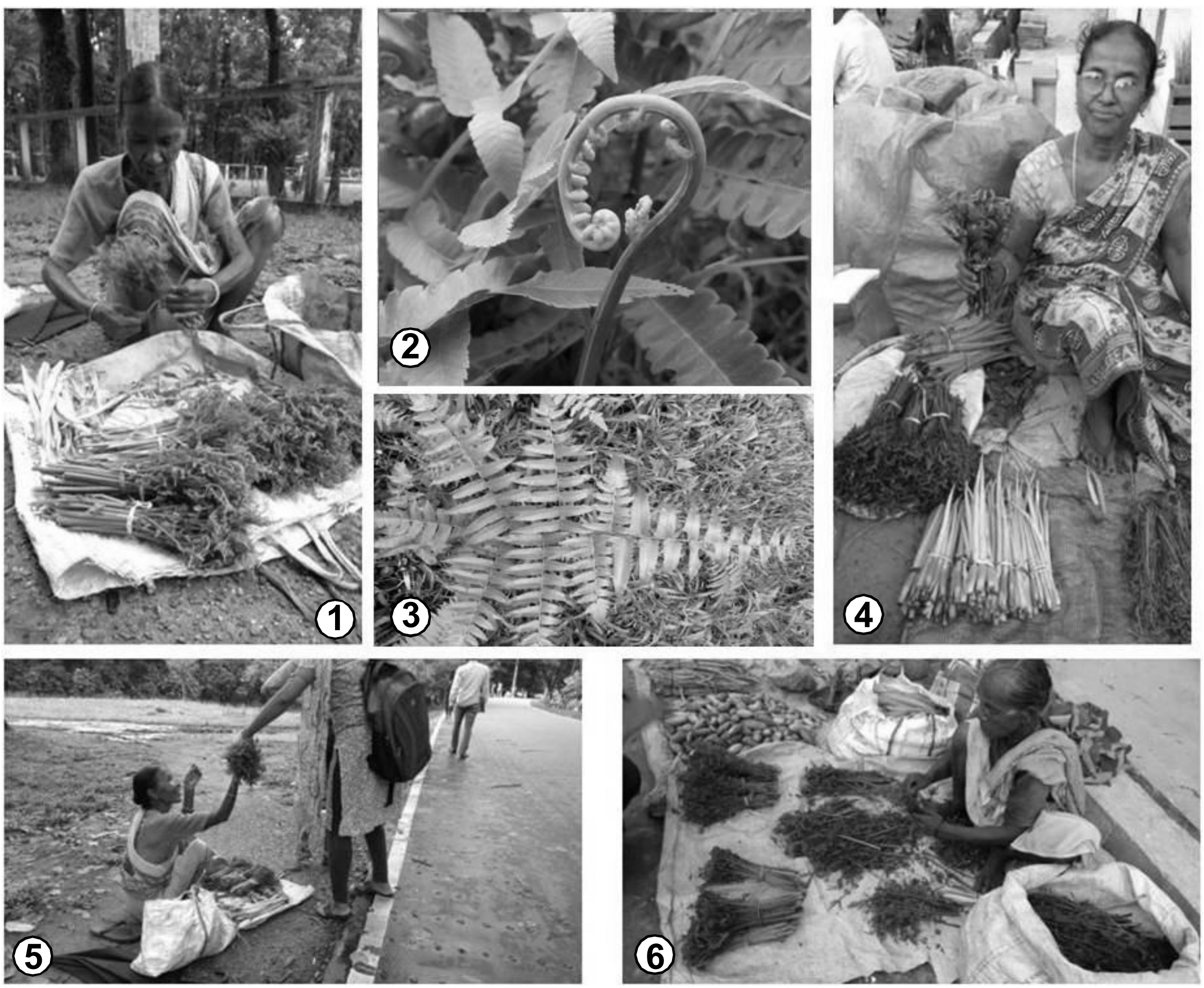

Plate: 1. Making of 'Mutha' by a seller 2. Young frond (edible stage) of *Diplazium esculentum* 3. Mature frond of *Diplazium esculentum* 4. Selling of *Colocasia esculentum* flower bud along with *Diplazium esculentum* 5. Direct selling of *Diplazium esculentum* to passers-by by the Collector herself 6. Selling of *Diplazium esculentum* fronds in a local market

Table 1: Uses of *Diplazium esculentum* (Athyriaceae) by different communities across India.

Community	Place	Parts used	Uses	Reference
Mishing, Bodo, Sonowal Kachari, Rabha	Poba Reserve Forest, Assam	Leaves, whole plant, rhizome	Tender leaves are cooked along with fruits of *Dillenia indica* and fish and consumed as vegetable; whole plant used as insecticides; decoction of rhizome used for haemoptysis and cough	Pegu *et. al.*, 2013
Santal, Kol, Bhumija, Bhuyan, Mahalis, Sounti, Saharas	Similipal Biosphere Reserve, Orissa	Root	20 gm fresh root is boiled in 1litre water till the volume reduced to 1/4th of the volume. 3ml of this decoction is taken with 2 ml honey for the cure of spermatorrhea	Rout *et. al.*, 2009
Mishing	Kaziranga National Park, Assam	Leaves	Used as vegetable, used essentially in the religious ceremony of the dead persons	Kutum *et. al.*, 2011
Bharia, Gond	Madhya Pradesh	Rhizome	Used as tonic	Rai, 1987
H'mar, Jaintia, Riang, Chorai, Hrangkhol, Mizo, Vaiphei paite, Karbi, Naga and *Kuki*	Cachar district, Assam	Young fronds	Used as vegetable, which stimulates digestion	Das *et. al.*, 2008
Meiteis, Nagas, Kukis	Manipur	Young fronds	Young fronds taken as vegetable	Yumkham, 2011
Murah, Bhumij, Santal, Bagti, Koll	Assam	Young fronds	Young fronds taken as vegetable	Sen and Ghosh, 2011
Todas, Kotas, Kurumbas, Irulas, Paniyas, Kattunayakas	Western Nilgiris, Tamil Nadu	Fronds	Raw young fronds are taken as vegetable, also cooked	Ramachandran and Udhayavani, 2013
Rabha	Assam	Tender leaves	Boiled and fried leaves are eaten as vegetable	Das and Teron, 2014
Reang	Tripura	Young fronds	Boiled with salt and taken to maintain good health	Shil and Choudhury, 2009
Adi	Arunachal Pradesh	Young frond	Boiled fronds are used in treatment of laxative	Kagyung *et. al.*, 2009

Beside, providing the basic needs, this fern have got considerable anti-inflammatory and anti-hepatoprotective activities (Nair *et al.*, 2015) and also significant cytotoxic, anti-microbial, antioxidant properties (Akter *et al.*, 2014). However, a study revealed, even after cooking, this fern may induce infertility to the male reproductive system (Roy and Choudhuri, 2015). Another study reported that daily consumption of the fern was found to elevate the risk of esophageal cancer (Somvanshi *et al.*, 2006).

Conclusion:

It is observed from our study that, *Diplazium esculentum* is having diversified uses. Even though it is marketed regularly by the local ethnic people and directly related with their socio-economic condition, there is no record for its cultivation. Therefore, it is suggested that it should be cultivated for its high demand to safeguard the natural vegetation.

Acknowledgement:

The authors are grateful to UGC, New Delhi for financial support.

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Available online at www.ewijst.org

ISSN: 0975-7112 (Print)
ISSN: 0975-7120 (Online)

Environ. We Int. J. Sci. Tech. 14 (2019) 25-36

Environment & We An International Journal of Science & Technology

A New Concept in Organic Farming: Efficacy of Brassinosteroids as Foliar Spray to Ameliorate Growth of Marigold Plants

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Article history:
Received 11 June 2018
Received in revised form
11 December 2018
Accepted 20 December 2018
Available online
31 January 2019

Keywords:

Epibrassinolide;
Foliar spray;
Organic farming;
Tagetes erecta

Abstract

Organic farming an alternative agricultural system, strives for sustainability and relies on use of natural fertilizers and soil amendments. The commonly used natural fertilizers such as compost, green manure, and bone meal have yielded positive results in a wide range of crops. In a global trend of Good Agricultural Practices (GAP), integrated methods are being adopted for raising crop plants and also flowers like lilies and carnations. Such practices have become important as chemically grown plants be it fruits, vegetables or flowers impact consumer health. These bio-safe methods help manage soil, control pests and provide post- harvest care.. It is therefore, imperative that we find more natural products that can be used as fertilizers and help raise crops organically. In one such attempt Epibrassinolide (EBL), a synthetic hormone belonging to Brassinosteroid group of plant hormones (other being homobrassinolide) was used as foliar spray. *Tagetes erecta* (L.) commonly known as marigold was sprayed with 24-Epibrassinolide at concentration of 10^{-8} M at 15-days stage and later at interval of another 15 days the second spray was given. Ameliorative effect of EBL on growth and photosynthetic parameters was studied and EBL emerged as a non-toxic, and eco-friendly foliar spray with a potential to increase both quality and yield of marigold.

Introduction

After investigating ten possible cases of pesticide poisoning among Miami florists in 1979, the American Journal of Public Health recommended that safety standards for residual pesticides on cut flowers to protect both florists and consumers should be implemented (https://www.greenamerica.org/green-living/say-it-organic-flowers), During 1980´s a wide variety of micronutrient ‘chelates’ and ‘complexes’ (e.g. synthetic chelates using EDTA, glucoheptonates, polyols, amino-acids, or lignosulphonates, among many other types) were offered as an alternative to the application of inorganic compounds (Fernandez *et al*., 2013). It is now shown that chelated micronutrients for foliar applications are often more effective than trace elements from inorganic sources. This may be largely because chelates not only guarantee the availability of micronutrients, but also facilitate absorption of the trace elements by the leaves (https://micronutrients.akzonobel.com/applications/foliar/). The proposition that stomata could contribute to the foliar penetration process was assessed by Eichert and co-workers at the end of the 1990’s and subsequently validated (Eichert *et al*, 1998; Eichert and Burkhardt, 2001; Eichert and Goldbach, 2008; Fernández and Eichert, 2009).

Though foliar application is a lucrative option but it is important to understand that such applications are not practical for nutrients that plants require in large amounts, such as nitrogen, phosphorous, and potassium. However, micronutrients can be easily administered through foliar application. At present a wide spectrum of foliar sprays are available, some are commercial while others are simple and can be prepared at home (Figure 1). Foliar sprays have certain limitations such as (a) they cannot be practiced in xerophytic plants with special anatomy to protect water losses (b) spray drift is another limitation (De Schampheleire *et al*., 2008) and (c) in perennial high-value crops, foliar fertilizers must be applied during the period of highest nutrient demand since soil supply and root uptake may be inadequate to meet demands even with adequate soil-applied fertilizer (Fernandez *et al.,* 2013).

In a study, nitrogen sufficient commercial 'Washington' navel orange trees were subjected to winter application of foliar urea just prior to or during flower initiation. Increased yield (p 0.05) was observed in three successive years without a reduction in fruit size (Ali *et al*., 1993). Carvalho (1994) observed that foliar application of KNO_3 at the rate of 0.3% brought best improvement in various growth parameters of Rangpur line seedlings (*Citrus limonia* cv. Cravo). In another study, Quin *et al*. (1996) found decreased leaf drop and increased fruit weight, fruit yield, soluble solids, juice content, ascorbic acid and total acid contents in 16 year old Eureka lemon tree when sprayed with 10% KCl. In Maize (cv. Jubilee) single foliar spray of 0.1M phosphate salts solution was applied to the upper surface at the five-to six-leaf stage, 2− 4 h before inoculation with *Puccinia sorghi.* This not only resulted in increased growth but also induced systemic resistance against common rust (Reuveni and Reuveni, 1998). Brassinosteroids (BRs), a class of steroidal phytohormones have pleiotropic effects on wide range of physiological responses such as growth, germination, flowering, abscission, and senescence. BRs are also known to confer resistance to plants against biotic and abiotic stresses (Ali *et al*., 2007). According to Rao *et al*. (2002) BRs comprise a specific class of phytohormones which possesses a significant growth promoting activity. BRs such as 24-

epicastasterone and 24-epibrassinolide promote root elongation up to 50% in wild-type plants of *Arabidopsis* (Müssig *et al*., 2003). Swamy and Rao (2006) studied the effect of 24-epibrassinolide and 28-homobrassinolide on an ornamental plant- *Pelargonium* sp. (geranium) bourbon type and observed better root and shoot growth. Youssef and Talaat (1998) also reported increase in the growth parameters in lavender by exogenous application of brassinosteroids.

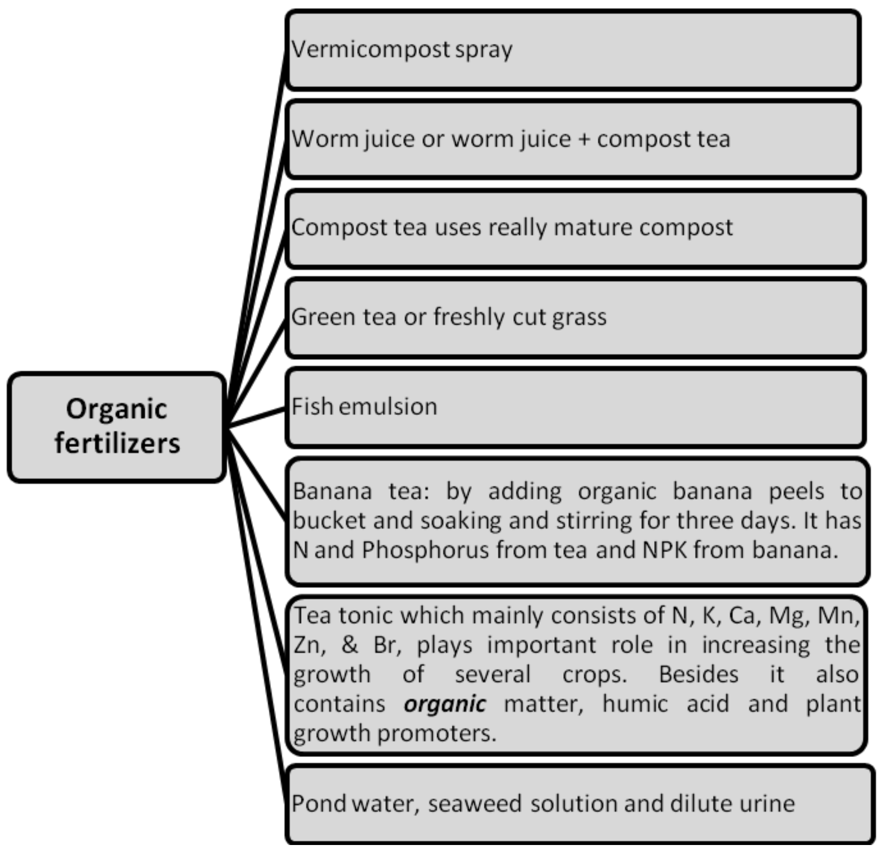

Figure 1 Types of organic fertilizers that easily prepared and administered while raising a kitchen garden.

Ameliorative effect of BRs on various plants growing under stress from salinity, drought and heavy metals are well known (Divi *et al* 2010, Cannata *et al* 2015). There remains little doubt if BRs are not able to bring about increase in growth and crop yield under stressful conditions. Similar data is also available on horticultural crops (Kang and Guo 2010). However, studies with BRs on flowers (and therefore in Floriculture) are recent. Kumar and Raju (2008) and Padamlatha *et al* (2013) have reported increase in yield of *Gladiolus* with BR application. It however, remains to be investigated if BRs can be recommended as components of commercial foliar sprays. Data is also lacking on the combinations of hormones which can give best results with BRs. Work on *Gladiolus* has indicated TIBA, GAs and CPPU combination to be effective in increasing growth and yield. It would also be worthwhile to increase anthocyanins, carotenoids and flavonoids concentration in flowers, as it would mean increase in quality of the produce. *Tagetes* L. is an annual herb that grows to about 30 cm in height. The bright orange to yellow flowers have been since ages used in religious ceremonies. They are also ground and used as chicken feed to enhance the characteristic yellow color of chicken skin and egg yolk. The pinnate leaves are a source of oil against skin problems. In foods and beverages, *Tagetes* is used as a flavor component. With a great importance attached to the plant, attempts are being made to increase quality of the produce. An experiment was therefore designed to investigate the potential of EBL in enhancing growth and yield of the plant through a sustainable method. The present investigation is only one of the few studies carried out with BR as foliar spray to increase growth and yield of a plant whose flowers are of great commercial potential.

Material and Methods

Preparation of hormone: Epibrassinolide (EBL) was purchased from Sigma Aldrich, Delhi. Stock solution (10^{-4} M) of Epibrassinolide (EBL) (Sigma Aldrich, Delhi, India) was prepared by dissolving the hormone in ethanol following protocol of Hasan *et al.,* (2011). The concentration (10^{-8} M) of EBL was prepared by dilution of stock in 0.5% Tween-20 (used as surfactant) solution of distilled water. The hormone was sprayed with atomizer all over the aerial plant surface during morning hours.

Experimental site and treatments: The experiment was carried out in a split plot completely randomized block design in Botanical Garden, SGTB Khalsa College, Delhi, India during the months of December 2017-March 2018. Seeds of *Tagetes erecta* L. (African marigold) variety Pusa narangi were purchased from National Seeds Corporation, Indian Agricultural Research Institute, New Delhi. Procured seeds were sown on a raised bed of size 110×50 cm to ensure appropriate germination. The nursery was maintained with proper irrigation and periodical weeding; seedlings that started to emerge after a fortnight were watered with a fixed amount of water daily and were covered with net to protect them from predators. After a month, the seedlings were transplanted into earthen pots of size 14x10 inches with one seedling per pot. Two series of six pots each were maintained where one series that was kept untreated, served as control and the other series sprayed with hormone was the treated one. After 15 days of transplantation (DAT) when the seedlings were about 20 cm long they were given first foliar spray of growth hormone 24-epibrassinolide (EBL). The second spray was at 30 DAT and each time a volume of 3ml 10^{-8} M EBL per plant was given. The control series was sprayed with distilled water. The treatments were named as follows:

1. Control (Pusa narangi) (PN)
2. PN+ Sprayed with EBL

There were six pots for each treatment and measurements were taken individually from each of the pots and data was pooled for statistical analysis.

Concentration of photosynthetic pigments: The chlorophyll and carotenoids concentration of leaves was determined by the non-maceration method using dimethylsulphoxide (DMSO) modified after Hiscox and Israelstem, (1979). The concentrations of chlorophyll a, chlorophyll b and carotenoids were calculated according to Arnon (1949).

$$\text{Chlorophyll a (mg g}^{-1}\text{ fresh weight)} = \frac{12.7D_{663} - 2.69D_{645} \times \text{Volume}}{1000 \times \text{weight of the sample}}$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ fresh weight)} = \frac{22.9D_{645} - 4.68D_{663} \times \text{Volume}}{1000 \times \text{weight of the sample}}$$

$$\text{Carotenoids (mg g}^{-1}\text{ fresh weight)} = \frac{7.6D_{480} - 1.49D_{510} \times \text{Volume}}{1000 \times \text{weight of the sample}}$$

Where, D= optical density and Volume= final volume of aliquot

Vegetative growth parameters

Plant height: was recorded from both control and EBL sprayed 45 days old plants. Measurements were taken with help of metre scale and height of plants was expressed in centimetres (cm).

Number of leaves and leaf area: Number of leaves was counted from both control and EBL sprayed 45 days old plants. For measurement of leaf area, the leaves were harvested and immediately traced on tracing paper and transferred on graph paper. The area was then calculated and expressed in cm^2.

Number of branches and internode length: The number of branches and internode length were recorded from both control and EBL treated plants. The internode lengths (third internode from below the apex) were measured with thread and metre scale and expressed in cm.

Reproductive growth parameters

Number of buds: The number of buds were counted from both control and EBL sprayed series at two stages. The first counting was done when plants were 60 days old and second counting was done at the time of final harvesting.

Number and diameter of flowers: The observations for number of flowers and diameter were taken at three different intervals from both control and EBL treated plants. Initial measurement for number of flowers was taken from 65 days old plants when the first flush of flowers was observed. The diameter of flowers was measured with help of thread and scale. The plants were tagged so as to keep a check that next observation was made from the same plant. The second and third readings were taken from 75 and 85 days old plants respectively.

Fresh weight and dry weight: Fresh and dry weight was taken at the time of final harvest from 90 days old plants. The fresh weights of plants were recorded immediately after the harvest using a physical balance (Sartorius, Germany). For recording dry weight, the plants were dried in oven at 60ºC till there was no change in weight in consecutive readings. Both the fresh and dry weight of plants was expressed in grams.

Statistical analysis: The data for both the series are given as mean ± standard deviation of six replicates. The significance of differences between means of the two treatments was calculated using the Student's t-test in Statistical Package for Social Sciences version 16 (SPSS, USA). The significance was checked at level of P<0.05 and statistically significant differences were shown with help of asterisk (*) in histograms and tables.

Results

Concentration of photosynthetic pigments: Exogenous application of EBL has significantly affected concentration of chlorophyll a and chlorophyll b. There was 60.9% and 29.03% increase in concentration of chlorophyll a and chlorophyll b in plants sprayed with EBL over control respectively (Figure 2). While no significant change was observed in concentration of carotenoids in control and EBL sprayed plants (Figure 2)

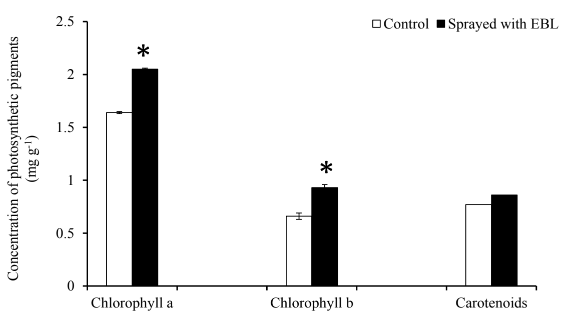

Figure 2. Effect of EBL spray on photosynthetic pigments in leaf tissue. Statistically significant differences were shown with help of asterisk (*)

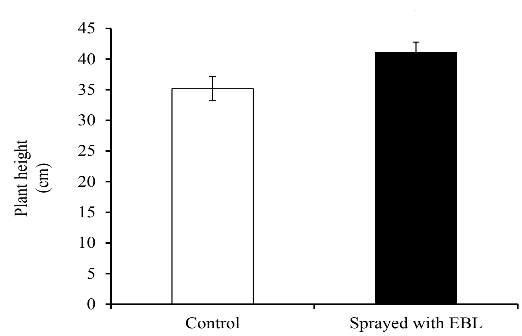

Figure 3. Effect of EBL spray on height of marigold plants

Vegetative growth parameters:

A significant difference in plant height was seen in plants sprayed with EBL as compared to control. Height of plants sprayed with EBL showed 14.57% increase over control plants (Figure 3). At the same time, numbers of leaves were also more in plants sprayed with EBL. EBL treated plants exhibited 12.33% increase in leaves as compared to control plants (Figure 4a). Further differences were seen for leaf area in plants that were sprayed with EBL. Significant increase of 39.45% was observed in leaf area for EBL sprayed plants as compared to control (Figure 4b). The marigold plants sprayed with EBL had significantly higher number of branches and internode length compared to plants without any spray. The number of branches significantly increased by 15.81%, while internode length increased by 9.73% in plants sprayed with EBL over control (Figure 5a and b).

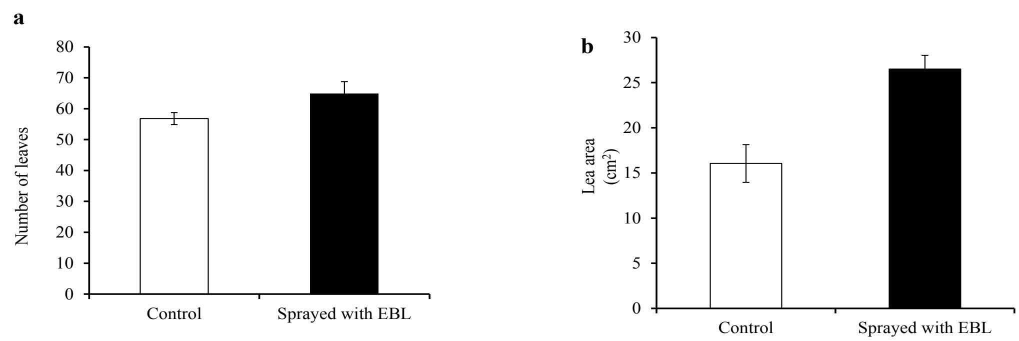

Figure 4 (a & b). Effect of EBL spray on (a) number of leaves; (b) leaf area of marigold plants.

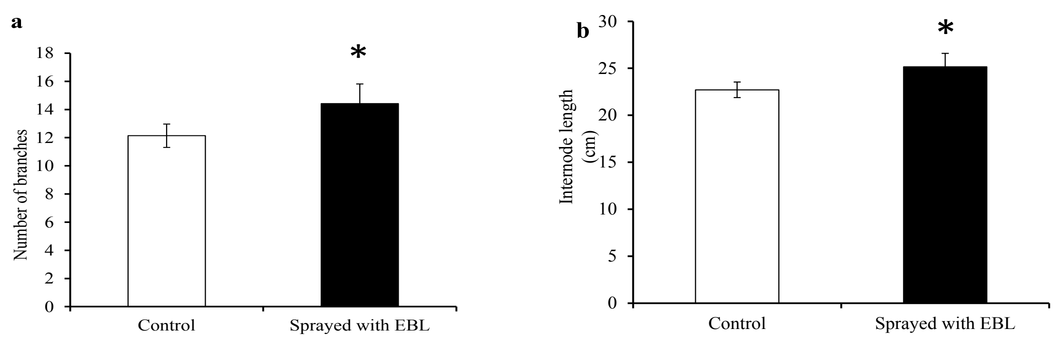

Figure 5 (a & b). Effect of EBL spray on (a) number of branches; (b) internode length of marigold plants. Statistically significant differences were shown with help of asterisk (*)

Reproductive growth: Number of buds was higher in plants sprayed with EBL as compared to control plants in both of the sampling stages (i.e. onset of reproductive phase and onset of senescence). Higher number of flowers was seen in EBL treated plants as compared to control in all the three stages of harvesting (Table 1). With each harvesting subsequently, the number flowers increased but the major difference was observed during third stage of harvesting between EBL treatment and control. There was increase in diameter of flower in EBL treated plants as compared to control in first (0.36%) and second (0.17%) stages of harvesting. However, slight decrease in diameter was observed during third stage of harvesting.

Table 1. Effect of exogenous spray of Epibrassinolide on number and diameter of flowers on three harvestings

Parameter	**Number of flowers**		**Mean diameter of flowers**	
	Control	**Sprayed with EBL**	**Control**	**Sprayed with EBL**
Ist harvest	6	7	4.7	6.1
IInd harvest	5	7	4.1	4.8
IIIrd harvest	13	16	3.7	3.5

Fresh weight and dry weight: Significant differences were observed in both fresh weight and dry weight of plants sprayed with EBL and those of control plants. Plants treated with EBL showed 17.03% and 27.2% higher fresh and dry weight respectively over control plants (Figures 6 and 7).

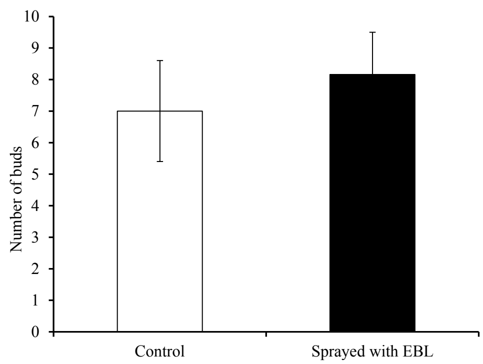

Figure 6. Effect of EBL spray on number of buds

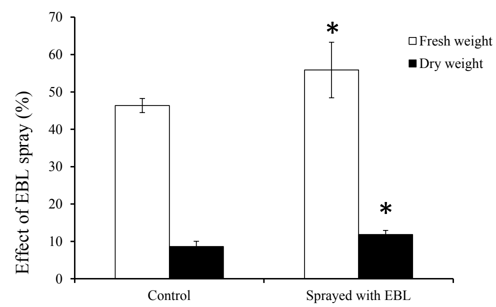

Figure 7. Effect of EBL spray on fresh and dry weight. Statistically significant differences were shown with help of asterisk (*)

Discussion

Problems associated with the use of synthetic chemicals for crop protection and weed control have been reviewed worldwide. It is known that with constant use of these chemicals - pests and weeds become resistant. The issues of human health and environmental pollution also cannot be ignored. Therefore organic fertilizers are increasingly becoming important with environmentalists, farmers and the consumers. Gradually the agro-chemicals are being replaced by fertilizers made out of many kinds of agricultural wastes such as animal dung and plant residues (Larptansuphaphal and Jitumroochokchai, 2009). In the present scenario of rising concerns of 'safe and organic' crops, foliar feeding using natural and organic fertilizer is an important tool for the sustainable and productive management of crops.

The foliage of plants exposed to EBL supplement exhibited enhanced photosynthetic pigments concentration that is in support of other studies (Ali *et al*., 2007;

Hasan *et al*., 2008). This is in compliance with earlier reports indicating the BRs-induced impact on transcription and translation involved in the expression of specific genes required for synthesis of biosynthetic enzymes involved in chlorophyll formation (Bajguz, 2000). In the present study the concentration of chlorophyll a (chl a) showed a better result as compared to chlorophyll b (chl b). The reason may be that application of BRs results in slowing down the rate of chlorophyll a degradation (Hola, 2011). There is also an increase in the chl a/b ratio in EBL supplemented plants indicating that chl b was being degraded at a higher rate than chl a. The results support the findings of Fang *et al* (1998) who demonstrated that the first step in the degradation of chl b implies its conversion to chl a. According to Khripach *et al* (1999) increase could be due to the prevention of the loss of photosynthetic pigments by BRs. The primary function of carotenoids is to absorb light energy for the use of photosynthesis, protect chlorophyll from photodamage and eliminate ROS formed in cell during various metabolic processes (Minguez-Mosquera *et al*., 2002). Carotenoid is a non-enzymatic antioxidant thus protecting chlorophyll and cell membranes against ROS (Das and Roy choudhury, 2014). In the present study, carotenoids content was not much affected thus showing no significant impact of EBL on carotenoids biosynthetic pathways.

In the present investigation all studied vegetative growth parameters were enhanced in EBL treated plants as compared to control. Improved growth could be seen in terms of increased plant height, intermodal length, number of branches and leaves, and also leaf area. Müssig (2003) reviewed that BRs could influence branch formation (also, in present work) by modulating metabolic pathway and by altering the nutrient allocation. However, the EBL induced leaf area is in viewpoint with the findings of Zhiponova *et al*. (2013) that BRs influence cell division and subsequently the leaf area and internode size. The increase in the chl a and b content along with increase in leaf area, could possibly lead to the improvement in photosynthetic efficiency of EBL treated plants.

It was observed that hormone treated plants have higher fresh and dry weight than control. The obtained results can be explained through work of Khripach *et al* (1999) that BRs prevent the loss of photosynthetic pigments thus retaining the photosynthetic efficiency and in turn affects the biomass of the tissue (Stoeva *et al*., 2005). Cutler (1991) showed that BRs increased plant growth, root size and root and stem dry weight. Çağ *et al* (2007) has also depicted similar results in red cabbage and reported fresh weight in cotyledons increased in 0.001 and 0.1μM EBL. Braun and Wild (1984) reported increased shoot fresh weight manifested by the stimulation of both elongation and radial growth in mustard. Besides enhancing vegetative parameters, effect of EBL was also observed on the reproductive parameters in present investigation. Increased bud production, flower number and size were observed in EBL treated plants as compared to control. The present work is indicative of correlation between vegetative and reproductive growth parameters. Subsequently, better vegetative growth leads to increased number of bud formation and flower size in hormone treated plants. Ethylene plays an important role in the regulation and co-ordination of senescence in climacteric flowers and a sharp increase of this hormone evolution was found during flower maturation, opening and senescence **(**De *et al.* 2014)**.** Arteca and Arteca (2008) findings support that BRs have been found to regulate ethylene levels. These above mentioned findings related to ethylene can be correlated with the present work that EBL enhanced not only the number of flowers but also the size of flower perhaps under the influence of ethylene. The result was in corroboration to the efforts of Khripach et al. (2000) where Epin supplemented varieties of roses showed enhanced production of flowers. According to Ye *et al*. (2012) in *Arabidopsis* sp. BRs regulate the timings of flowering while Yu *et al*. (2008) observed that BRs enhance carbohydrate assimilation, allocation, control of aquaporin activities and also various developmental processes and organ differentiation. These aspects however need to be investigated in *Tagetes*.

Judicious combination of sustainable practices like biofertilizer application as well as growth regulator foliar sprays creates possibilities of manipulating flowering to a rational quantity, which would prove useful in commercial species like marigold. Present study proves efficacy of BRs in bringing significant effects on plant growth and development and therefore may be one of the components of foliar sprays or nutrient supplements.

Acknowledgements: The authors are grateful to the Science Research Scheme, SGTB Khalsa College, University of Delhi, for providing financial assistance. Thanks are also extended to Dr. Jaswinder Singh Principal, SGTB Khalsa College, University of Delhi, Delhi for providing necessary facilities to carry out the study. We also thank Dr. Pratibha Brahmi, Officer- in- Charge, Germplasm Exchange at National Bureau of Plant Genetic Resources, New Delhi for providing us the seeds of *Tagetes*.

Author's Contribution: *Inderdeep Kaur, Anjana Sagar and Piyush Mathur wrote and edited the manuscript. Inderdeep Kaur and Anjana Sagar has planned methodology and designed the work. Anirudh Mukerji, Sagar Dhama, Vibhav Singh and Jasmeen Kaur meticulously carried out the experiments and Anirudh Mukerji and Sagar Dhama also collected data. Piyush Mathur analysed the results and prepared the manuscript.*

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Indian Fern J. **36 :** 283-286 (2019) ISSN 0970-2741

THELYPTERIS TRIPHYLLA (SWARTZ) K. IWATSUKI [THELYPTERIDACEAE] - A NEW RECORD FOR THE FERN FLORA OF JALPAIGURI DISTRICT, WEST-BENGAL, INDIA

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(Received September 27, 2019; Revised Accepted October 20, 2019)

ABSTRACT

Thelypteris triphylla (Swartz) K. Iwatsuki is reported from the district Jalpaiguri of West Bengal. This species is rarely found and known only from two areas of the district. The detailed description, illustrations of the plant, stomata, scale, spores are provided to enhance the identification process.

Key Words : Fern, Stomata, Scale, Spore, Jalpaiguri.

INTRODUCTION

The genus *Pronephrium* C. Presl belongs to the family Thelypteridaceae, of about 68 species, of these – 8 species are reported from India (Dixit, 1984). Fraser-Jenkins *et al.* 2017 includes the genus *Pronephrium* within the large genus *Thelypteris.* According to Fraser-Jenkins *et al.* (2017) Family Thelypteridaceae consists of one genus, *Thelypteris* which is is represented in India by 83 species (Fraser-Jenkins (2016).

Thelypteris triphylla (Sw.) K. Iwats. (syn. *Pronephrium triphyllum*) was reported earlier from Dulkhajar (Indo-Nepal Border), Darjeeling District of West Bengal (Fraser-Jenkins *et al.*, 2017). During the present survey of Jalpaiguri district of ferns and fern-allies – a total number of 46 Pteridophytes is collected, out of these *Thelypteris triphylla* (Swartz) K. Iwatsukiis a new report from Raipur Tea garden (latitude 26^{0}36'20.14"N and longitude 88^{0}40'35.42"E) and Sipchu-Chapramari forest Range adjoining regions (latitude 26p 56’56.33” N and longitude 88p 51’40.06” E) in the month of December’18. The district Jalpaiguri is a transition area between Duars and Terai region. It is very close to Indo-Bhutan hills with 3044 KM^{2} total geographical area. The district has latitude of 26°32’36.52” N and longitude of 88°43’13.89”E with an average elevation of 180 meter above sea level. Geographically the district is situated in the northern part of West-Bengal. The average rainfall of this area is 3000 mm per annum.

Since the publication of Bengal plants by David Prain (Prain 1903) a lot of work has been done on Pteridophytes. In Eastern Himalayan region initial work on Pteridophytes is performed by Mehra and & Bir (1964); Sikdar *et al.* (1983); Ghosh *et al.* (2004); Paul (2016). The present study was done on foot hills and plains of Eastern Himalayan regions of Jalpaiguri district of West Bengal, India.

MATERIALS AND METHODS

After collecting the species were preserved in FAA for routine laboratory work.

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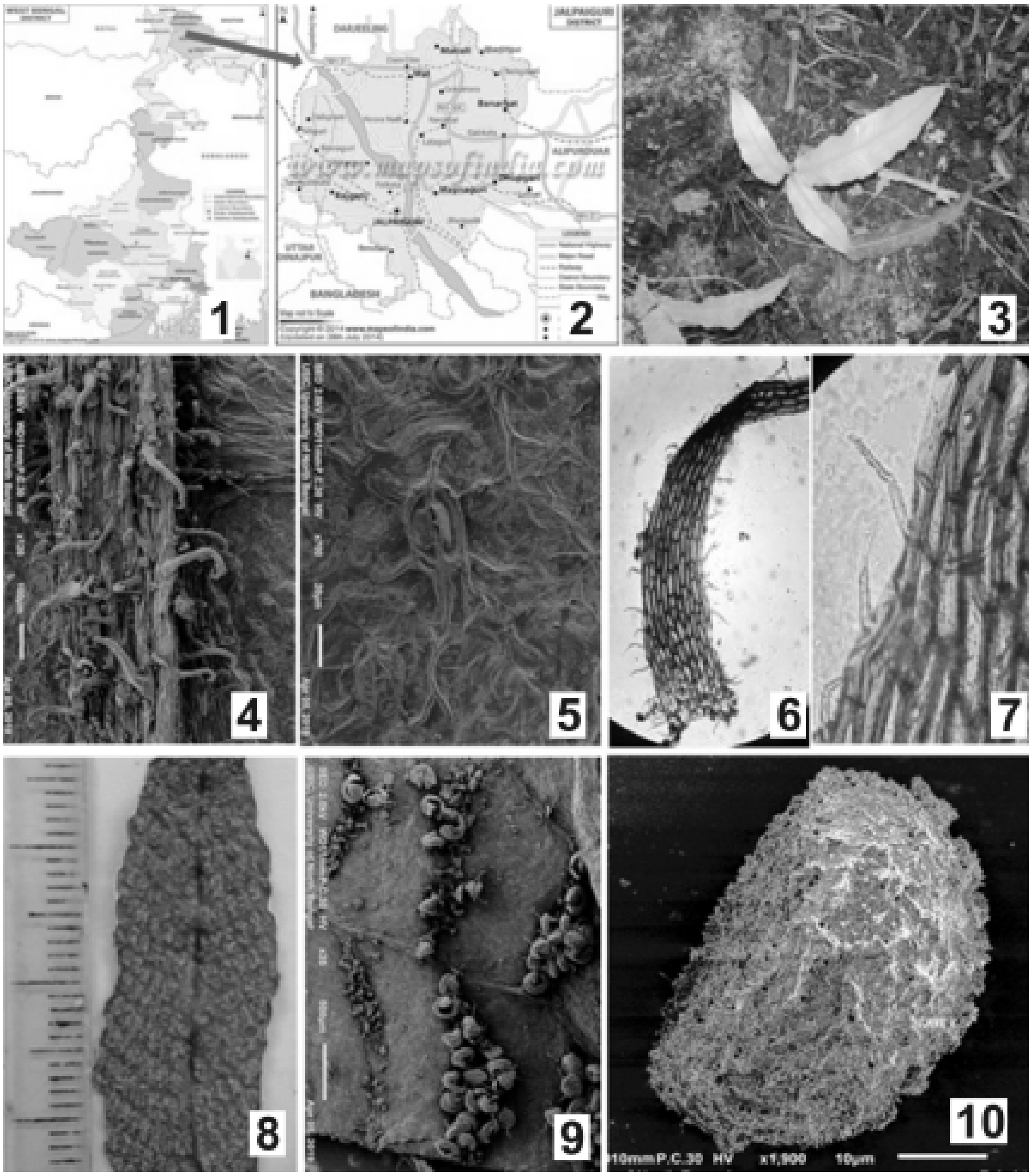
The herbarium of the said specimen was deposited at "NBU Herbarium" in NBU with accession no.10649. After proper examination and literature study (Chandra, 2000, Borthakur *et al.*, 2001, Ghosh *et al.*, 2004, Fraser-Jenkins *et al.*, 2017, and Singh & Panigrahi 2005) it was identified as *Thelypteris triphylla* (Swartz) K. Iwatsuki. Routine procedure is followed for morphological studies under simple and compound microscope for leaf epidermis, scale and petiole. Epidermal clearing was made by heating a piece of leaf dipped into 5% NaOH at 55°C in a test tube. It was washed thoroughly in water and bleached by sodium hypochlorite solution (10%). The cleared leaf material was now stained by 1% Aqueous-Safranine, dehydrated and made permanent for further observations. Spore morphology is based on acetolysis preparation of spore (Erdtman, 1952 & 1969). For SEM (JEOL microscope Model No. JSM-IT100) study dry spores were mounted on carbon tape of brass stub.

DESCRIPTION

Thelypteris triphylla (Sw.) Iwats.

Thelypteris triphylla (Sw.) K. Iwats. In mem. Coll. Sci. Univ. Kyoto B, 31: 190. 1965; Syn.: *Meniscium triphyllum* Sw. in Schrad. J. Bot. 1800. (2): 16. 1801; *Phegopteris triphylla* (Sw.) Mett., Lechl. 2: 27. 1859; Bedd., Ferns South India, t. 56. 1864 & Handb. Ferns Brit. India: 397. pl.231. 1883; *Nephrodium triphyllum* (Sw.) Diels, Nat. Pflanzenfam. 1(4): 178. 1899; *Dryopteris triphylla* (Sw.) C. Chr., Ind. Fil.: 298. 1905; *Abacopteris triphylla* (Sw.) Ching in Bull. Fan Mem. Inst. Biol. 8: 241. 1938; *Cyclosorus triphyllus* (Sw.) Tardieu-Blot ex Tardieu-Blot et Ching in Notul. Syst. 7: 77. 1938; Copel., Gen. Fil.: 143. 1947, et Fern Fl. Philip.2: 371. 1960; *Pronephrium triphyllum* (Sw.) Chandra in Bull. Bot. Surv. India.13: 274. 1971; *Pronephrium triphyllum* (Sw.) Holtt., Blumea, 20(1). 122. 1972; Nayar in Nayar and Kaur, Comp. Bedd. Handb.: 212. 1974; Sledge in Bull. Br. Mus. Nat. Hist. (Bot.) 8: 47. 1981; Baishya & Rao, Ferns & Fern-allies Meghalaya, 86. 1982; Kaur & Chandra in New Botanist 12: 101. 1985; Jamir & Rao, Ferns Nagaland, 261. 1988; Manickam & Irudayaraj, Pterid. Fl. West. Ghats-S. India, 185.t.140.1988; *Pronephrium nakaikei* R. D. Dixit & Bal Krishna, *Indian Fern J.* 7(1 & 2): 1-4, t. 1-3.1990 (as "nakaikeium"). **Rhizome** long, 0.5 cm thick, creeping, slender covered with scales, root hairs blackish-brown in colour; **Scales** (3 ×1 mm) linear-lanceolate (PLATE I, Fig.6), apex acuminte, apex with deciduous gland, margin entire, hairy (PLATE I, Fig.7), clathrate scales present at the base of the petiole, dark-brown in colour; **Stipes** in sterile lamina 6-20×0.2-0.3 cm, it is sometime longer in fertile lamina, slender, grooved above, flattened below; green to brownish in colour, covered with dense hair; **Lamina** (7-18×5-11 cm), dark-green at adaxial side and light green at abaxial side, ovate in shape trifoliate; lateral pairs of pinnae 3-10×1.5-2.5 cm, lanceolate, opposite, petiolate (0.5-2 cm), apex acuminate, attenuate at the base, undulate to serrate margin, costules & veins distinctly prominent abaxially, veinlets obliquely spreading and parallel, costules are dense hairy (PLATE I, Fig.4); all veins of adjacent costules meet at the centre; texture subcoriaceous;

PLATE I



Figs. 1 & 2. Location site of the plant; **3.** Habit of the plant; **4.** Costule with hair; **5.** SEM of stomata; **6.** A scale, ×22; **7.** Margin of scale, ×90; **8.** Fertile lamina; **9.** SEM of Sori orientation through veinlets; **10.** SEM of a spore stomata copolocytic (PLATE I, Fig.5); lamina adaxially glabrous but abaxially with hooked hairs along costae and veinlets, intercostal areas with scattered hairy hooked; **Sori** developing along the veinlets of the lamina, oblong to slightly curved (PLATE I, Fig.9), ex-indusiate, coenosori; **Sporangia** with 2 hairy hooked; **Spores** 38×22 µm,monolete, reniform, regulate (PLATE I, Fig.10), exine spinulose.

DISTRIBUTION

India : West-Bengal, S. India, Sikkim, Assam, Arunachal Pradesh; **Sri Lanka**, **Myanmar**, **Thailand**, **Malaysia**, **China**, **Japan**, **Taiwan**, **Philippines**, **Australia**.

DISCUSSION

The above specimen (Plate I, Fig. 3) was found less in population in respect to other fern genera (out of 15 in that area). During the present work it was observed urbanization, over population and construction of roads might be the cause of destruction of the natural habitat of plants along with this fern. The flora needs well exploration and identification of the ecologically and ethno-botanically important ferns and requires maintaining some conservation strategies.

ACKNOWLEDGEMENTS

Authors are thankful to Prof. Radhanath Mukhopadhyay and Dr. Jaideep Mazumder for their valuable suggestions and identification of the specimen. Authors also thankful to the Head, Department of Botany, University of North Bengal, Siliguri for providing necessary facilities. We also convey our thanks to the Head and all other faculty members, Department of University Science Instrumentation Centre, NBU, Siliguri for their kind cooperation during the SEM study.

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NBU Journal of Plant Sciences

Vol. 10, No. 1, March 2016 p. 31-44
ISSN No. 0974-6927

Review Article

Nitric oxide and calcium signalling in plants under salinity stress and their crosstalk

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Abstract

Salinity is considered as one of the major factor affecting the crop production throughout the world. The oxidative stress induced by salinity can retard plant growth and yield as major part of energy is wasted on conserving water and improving ionic balance. The free radicals produced during stress are considered to be a major factor for most of the damages as these free radicals attack vital biomolecules such as lipids, protein and carbohydrates which are the basic requirement of almost all physiological and developmental processes. Understanding the mechanism of stress tolerance along with the involvement of important signalling molecules in stress signalling network is essential for crop improvement. Likewise, the two signalling molecules nitric oxide and calcium ion have been reported to be actively involved in upregulation of various stress response mechanisms thus indicating the existence of a possible cross talk among these molecules and other associated pathways. In this review, emphasis was given on the impact of salinity and oxidative stress mediated damages on plant system. Additionally, the role of nitric oxide and calcium ion as signalling molecules in response to stress signals and their implication in mitigation of salinity stress has also been discussed.

Keywords: Calcium ion, Free radicals, Nitric oxide, Salinity, Signalling.

Introduction

Salinity is considered as one of the major factor affecting the crop production throughout the world. Salinity either in water or soil represents one of the major abiotic stresses especially in arid and semi-arid regions, which can severely limit the agricultural production (Shanon, 1998). High concentration of salt creates ionic imbalance and hyper osmotic stress in plant system which consequently leads to oxidative damages. Such drastic changes in plant system cause retardation of growth, molecular damages, membrane disruption and even death. For the plant to be tolerant to salinity stress: their homeostasis must be re-established along with detoxification mechanism must be boosted (Zhu, 2001). Most of the cellular damages caused by salinity are usually associated with ROS mediated oxidative stress (Parida and Das, 2005).

Nitric oxide and calcium both are considered as highly versatile signalling molecules. Various literatures have reported the significant involvement of both of these molecules in wide range of physiological and developmental processes in plants. Additionally, these molecules have found to mitigate the adverse effect of varied environmental stresses including salinity (Wilson *et al.*, 2008; Sirova *et al.*, 2011; Lecourieux *et al.*, 2006).

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Effect of salinity on plant system

The two major consequences of salinity on plant system are osmotic stress and ionic toxicity; these physical conditions affect all other physiological, biochemical and developmental processes in plants (Yadav *et al.*, 2011). High salt content in the substratum creates rise in osmotic pressure of the substratum thus, affecting the water uptake capacity of plants. Furthermore, decrease in the turgor pressure of the plant cells cause closing of stomata which leads to reduced carbon fixation but increase in ROS production. These highly reactive and unstable free radicals disrupt various cellular processes by damaging the major biomolecules like lipids, proteins, and nucleic acids (Parida and Das, 2005). Ionic toxicity is the physiological state in which the equilibrium of ions is disturbed which causes perturbation in cellular metabolism and processes. High concentration of sodium ions at the surface of the root disrupts plant nutrition by inhibiting both K^+ uptake and enzymatic activities within the cell (Aslam *et al.*, 2011). Potassium is an important nutrient which regulates huge number of enzymes activities associated with various major pathways (Kader and Lindberg, 2010); on the other hand, sodium ions inhibit the activity of enzymes. Na^+ is a cation almost similar to K^+, for this reason Na^+ can cross the cell membrane without much disturbance (Parida and Das, 2005). As suggested by Rodriguez-Navarro, (2000) optimum concentration of K^+ required is 100-200mM in the cytosol and the concentration of cytosolic Na^+ excess of 10mM creates stress environment in the system. The oxidative stress induced by salinity can retard plant growth as major part of energy is wasted on conserving water and improving ionic balance (Kader and Lindberg, 2010).

Strategy for prevention of Na^+ toxicity in plants

In order to overcome salt stress, plants have developed different strategies for their survival. For instance, for combating Na^+ toxicity most of the glycophytes depend on restriction of Na^+ intake, but this strategy is successful to some extent only because of the electronegative environment in inner cellular system. Additionally, the cation transporters are fairly permeable to Na^+, therefore the constant influx of Na^+ along the electrochemical gradient is not terminated completely (Amtmann *et al.*, 1999). But interestingly, halophytes overcome this ion toxicity by coupling the uptake of ions via roots with the compartmentation of ions into cellular vacuoles (Hasegawa *et al.*, 2000; Blumwald *et al.*, 2000).

Concept of free radicals

A free radical is defined as a molecular species which is capable of independent existence and posses an unpaired electron in its outermost atomic orbital. This unpaired electron results in presence of certain common properties that are shared by most of the radicals. These free radicals are highly unstable as well as highly reactive. They have the capability to either donate an electron or accept an electron from other molecules, therefore altering their native properties (Cheeseman and Slater, 1993; Lobo *et al.*, 2010). The free radicals generated from oxygen are called reactive oxygen species (ROS) and those from nitrogen are termed as reactive nitrogen species (RNS). ROS includes various forms of activated oxygen molecules, such as superoxide ($O^{2\bullet -}$), hydroxyl ($^{\bullet}OH$) and peroxyl ($ROO^{\bullet}$), as well as non-free radicals hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Likewise, RNS includes nitric oxide ($NO^{\bullet}$) and nitrogen dioxide ($NO_2^{\bullet}$) and non-free radicals such as nitrous acid (HNO_2) as peroxynitrite ($ONOO^-$) (Halliwell, 1994; Chanda and Dave, 2009). These free radicals are generated under normal physiological conditions but become harmful when not being eliminated from the cellular systems. In fact, such imbalance between the production and elimination of reactive oxygen species in the cell system leads to a condition known as oxidative stress. After excessive accumulation, they attack vital biomolecules leading to cell damage and homeostatic disruption. The major targets of these free radicals are lipids, nucleic acids, proteins and carbohydrates (Aruoma, 1994). The formation of free radical is a consequence of both enzymatic and non-enzymatic reactions which occurs continuously in the cell system. Enzymatic reactions include those phenomena involved in the phagocytosis, respiratory chain, synthesis of prostaglandin also in the cytochrome P450 system (Lui *et al.*, 1999; Lobo *et al.*, 2010). Free radicals can also be produced in non-enzymatic reactions between oxygen and organic compounds as well as those initiated by ionizing reactions.

Importance of Nitric oxide signalling in plant system

Nitric oxide (NO) is an important signalling molecule, which has been known to participate in wide spectrum of regulatory functions in almost all stages of plant development (Wilson *et al.*, 2008; Sirova *et al.*, 2011). In the year 1975 the emission of NO from plants was first observed by Klepper in 1975, in soybean plants treated with herbicides (Klepper, 1979).

Plants not only react to the atmospheric or soil NO, but they are also able to generate NO via reduction of apoplastic nitrite (Bethke *et al.*, 2004) or by carotenoids in presence of light (Cooney *et al.*, 1994). The major production of NO in plants, however, is probably carried through the action of NAD(P)H-dependent nitrate reductase enzyme (Dean and Harper, 1988) which is also considered as an endogenous source of NO in plant system (Yamasaki *et al.*, 1999).

The synthesis of NO in animals is carried out by the enzyme nitric oxide synthase (NOS) via deamination of L-Arginine. But, there are no such genes in plant system including *Arabidopsis thaliana* that allow homology with *NOS* genes of animals (Gupta *et al.*, 2011). Among the photosynthetic members, only *Ostreococcus tauri*, an unicellular green alga was found to possess a NOS having a homology of only 45% with the human NOS (Foresi *et al.*, 2010). At present several pathways involved in NO synthesis in plant system are known, also some are assumed which are given in fig. 1. The biosynthetic pathway leading to the production of NO in plants might be either oxidative or reductive. The oxidative pathway is carried out by NOS like enzyme which also includes synthesis from polyamines. The reductive pathway is mediated by enzymes such as nitrate reductase (NR) and nitrite–NO reductase (Ni-NOR). Furthermore, this pathway includes xanthine oxidoreductase (XOR) in peroxisomes and cytochrome *c* oxidase (COX) that synthesizes NO from nitrite in mitochondria (Mamaeva *et al.*, 2015).

The application of exogenous NO to plants or cell cultures has revealed valuable information about the influence of this molecule on various physiological and biochemical processes. The summary of the functions NO associated with various physiological, biochemical and molecular processes is given in fig. 2. The earlier reports suggest that NO can mediate the biological effects of signalling molecules such as phytohormones. The biosynthesis of NO has been found to be induced by cytokinin in different plants and hence the possibility of involvement of NO in the cytokinin-induced programmed cell death process is proposed by Neill *et al.*, (2003). Likewise, it has been demonstrated that NO synthesis in cucumber roots is induced by auxin (Pagnussat *et al.*, 2003). Additionally, the interaction between both the gaseous molecules NO and ethylene in the maturation and senescence of plant tissues has been reported during plant development (Lamattina *et al.*, 2003).

The identification of the NO synthesis enzymes and the discovery of regulatory role of NO in the activity of specific proteins within sub-cellular compartments provided significant understanding of NO signalling at the molecular level (Hanafy *et al.*, 2001; Kone *et al.*, 2003; Stuehr *et al.*, 2004). Over the past decade, considerable progress has been made in understanding the mechanism of NO signalling in plants. NO modulates the activity of most proteins through nitrosylation and tyrosine nitration mechanism. The post translational modifications via nitrosylation as well as S-nitrosylation have been resulted in regulation of several plant proteins *in vitro* and also *in vivo* to some extent. The proteins which are the targets of NO include haemoglobin, cytochrome c oxidase, metacaspase 9, glyceraldehyde-3- phosphate dehydrogenase, and methionine adenosyltransferase (Besson-Bard *et al.*, 2008). Endogenous NO has been found to function as a calcium ion-mobilizing messenger by inducing the rise in cytosolic Ca^{2+} concentrations. The rise of cytosolic Ca^{2+} concentration further aid NO to modulate the protein kinases and channels involved in the signalling cascade, thus regulates important physiological processes such as stomatal closure, adventitious root formation an also the expression of defense genes (Garcia-Mata *et al.*, 2003; Lamotte *et al.*, 2006). In *Arabidopsis*, it was demonstrated that the production of NO by elicitors such as lipopolysaccharides is regulated by Ca^{2+} influx mediated by the cyclic nucleotide-gated channel (Ali *et al.*, 2007). The interplay of both NO and Ca^{2+} and their involvement in the plant acclimation during salinity stress also preventing the oxidative stress mediated damages and their adverse effects have been elucidated in fig. 4.

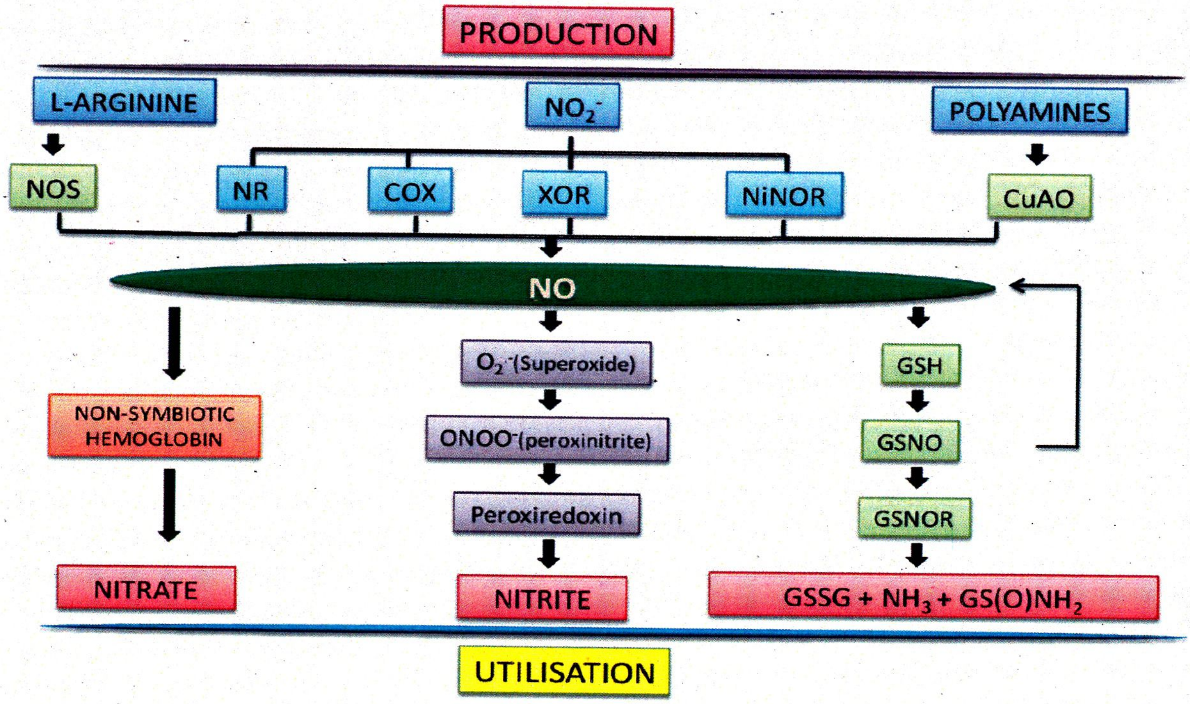

Fig. 1: Pathways involved in synthesis and utilization of NO in plant system (Mamaeva *et al.,* 2015).NOS: Nitric oxide synthase; NR: Nitric reductase; COX: Cytochrome oxidase; XOR: xanthine oxidoreductase; CuAO: Cu-amine oxidase; NiNOR: Nitrite-NO reductase; GSH: Reduced glutathione; GSNO: S-nitrosoglutathione; GSNOR: S-nitrosoglutathionereductase; GSSG: oxidized glutathione; $GS(O)NH_2$: glutathione sulfonamide.

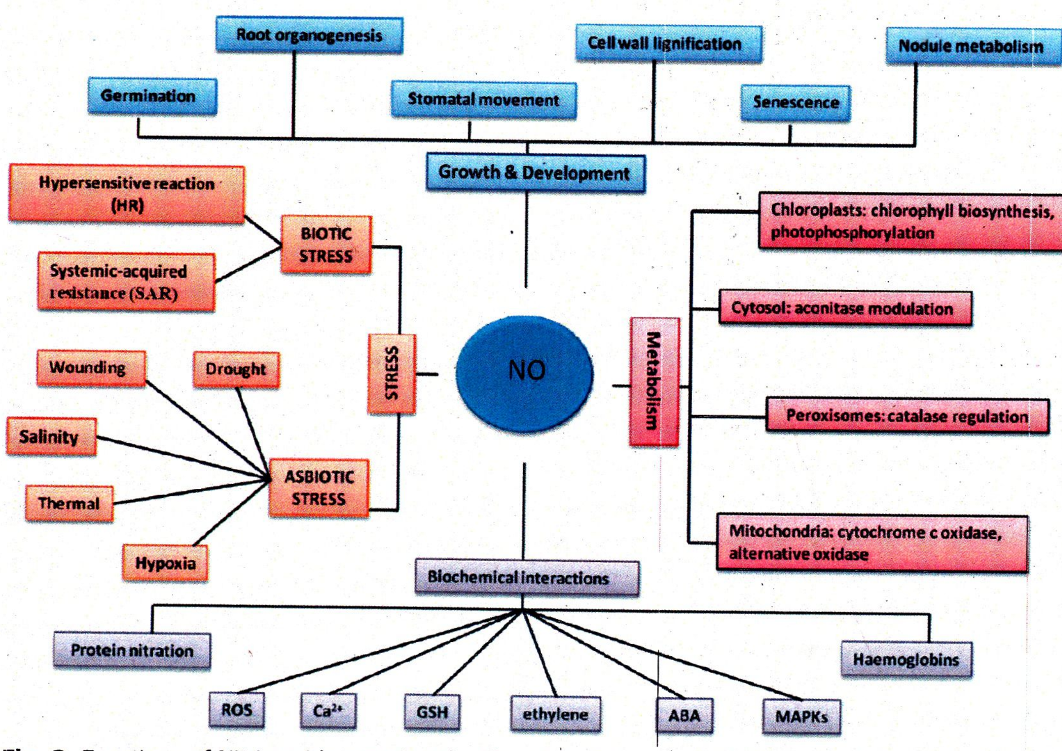

Fig. 2. Functions of Nitric oxide associated with various physiological, biochemical and molecular processes (del Rio et al., 2004).

Role of Calcium signalling in plant system

The calcium ion has been well established as a second messenger in several plant signalling pathways, conveying a wide range of stimuli to appropriate physiological responses. Ca^{2+} signals are considered as a core regulator of cell physiology and cellular responses of plants to the environment. Many extracellular and environmental signals including both abiotic and biotic factors, elicit change in the cellular level of calcium, termed as calcium signatures (Lecourieux *et al.*, 2006). This "Ca^{2+} signatures" represent a central mechanistic principle for stimulus-specific information in the cellular system. The channels, pumps, and carrier proteins serve as the mechanistic basis for generation of Ca^{2+} signals by modulating the flux of calcium ions among the sub-cellular compartments, cell and its extracellular environment (Dodd *et al.*, 2010).The disorders due to Ca-deficiency in plants have been considered to be very much harmful in horticulture sector commercially (Shear, 1975). Some of the diseases caused due to deficiency of calcium in plants are tip burn and brown heart in leafy vegetables, blossom end rot of tomato fruit, empty pod in peanut also structural weakness in cell wall. The Ca-deficiency generally occurs when there is unavailability of sufficient calcium in the developing tissues due to failure of calcium mobilization by phloem. On the other hand, presence of excess calcium in the substratum also creates a cytotoxic environment for plants. The excessive calcium reduces the germination rate of the seeds and also retards the plant growth rates (Shear, 1975; White and Broadley 2003). The other functions of calcium ion in the plant systems are elucidated in fig. 3.

Since the presence of higher calcium ion concentration is cytotoxic, a sub micromolar level of calcium ion is maintained by Ca^{2+}ATPases and H^{+}/Ca^{2+} antiporters in unstimulated cells (Sze *et al.*, 2000; Hirschi, 2001). These proteins maintain this optimum level by fluxing the extra cytosolic Ca^{2+} either to the apoplast or the lumen of vacuole or endoplasmic reticulum (Sanders *et al.*, 2002). There are other class of proteins which change their conformation or catalytic activity upon binding with the calcium ion and hence regulate the calcium signals. Also it has been reported that specific sensors and signals of calcium ion signatures regulated cellular responses to specific biotic and abiotic stimuli (White, 2000).

The proteins responsible for the perception and decoding of Ca^{2+} signals are present in the cytosol and nucleus of the plant cell. Several calcium sensors with different Ca^{2+} binding characteristics, subcellular localizations and signalling interactions comprises a toolkit that helps in decoding the information within Ca^{2+} signatures in the form of spikes or oscillations (Dodd *et al.*, 2010; Batistic and Kudla, 2012). Further these sensor proteins accordingly carry the processing of this information into respective alterations in cell function. Conceptually, plant Ca^{2+} sensor proteins that are functionally signalling components have been classified into sensor relays and sensor responders (Sanders *et al.*, 2002). The sensor responder proteins which include Ca^{2+}- dependent protein kinases (CDPK) combine both sensing function and responding function, regulated by calcium-binding proteins that often cause conformational changes (e.g., protein kinase activity) within a single protein. Consequently, these kinases mediate the information encoded in Ca^{2+} signals into phosphorylation events of specific target proteins. In contrast, sensor relay proteins such as calmodulin (CaM) and calmodulin like protein (CML) also contain multiple calcium-binding domains and undergo conformational changes with Ca^{2+} signals but lack the enzymatic function. Therefore, these proteins have to interact with other target proteins and regulate their activity for transduction of Ca^{2+} signal, which means they must undergo Ca^{2+}-dependent protein-protein interactions (Luan *et al.*, 2002). The calcineurin B-like (CBL) protein are another family of sensor proteins which lack the enzymatic activity hence belong to sensor relay proteins. However, their specific interaction is with a family of protein kinases designated as CBL-interacting protein kinases (CIPKs), so, CBL–CIPK complexes are considered as bimolecular sensor responders (Hashimoto and Kudla, 2011). CaM is highly conserved in all eukaryotic members, whereas

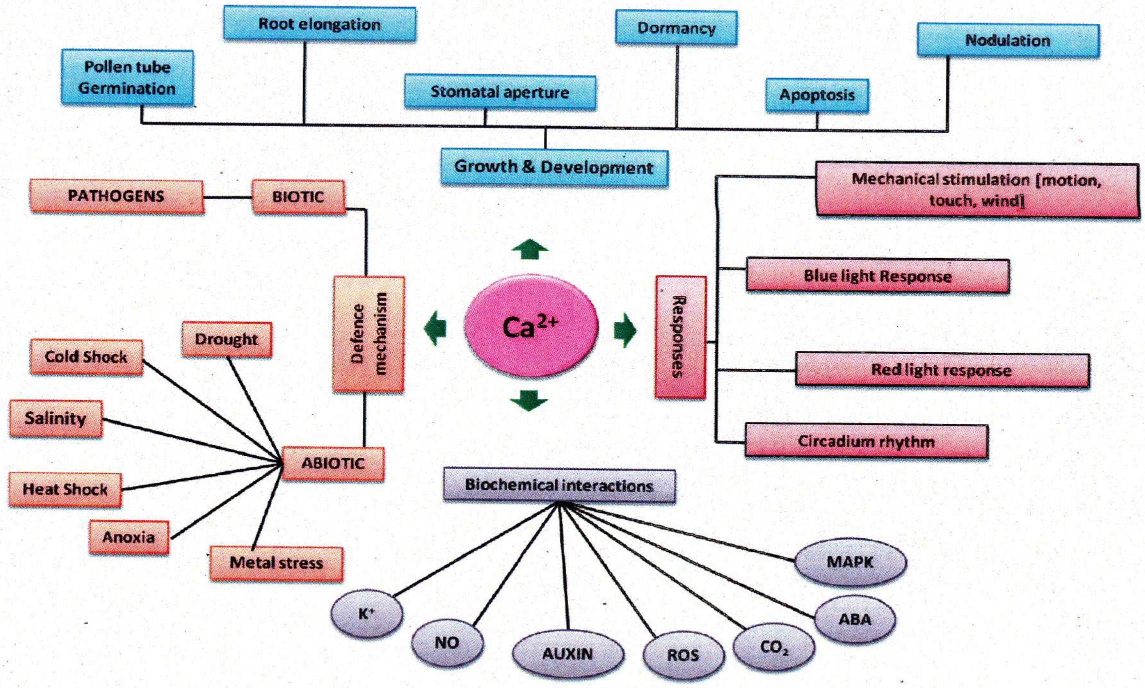

Fig. 3. Involvement of Ca^{2}+ signal in various physiological, biochemical and molecular processes in plant system (modified from White and Broadley, 2003; Leucouriex *et al.*, 2006)

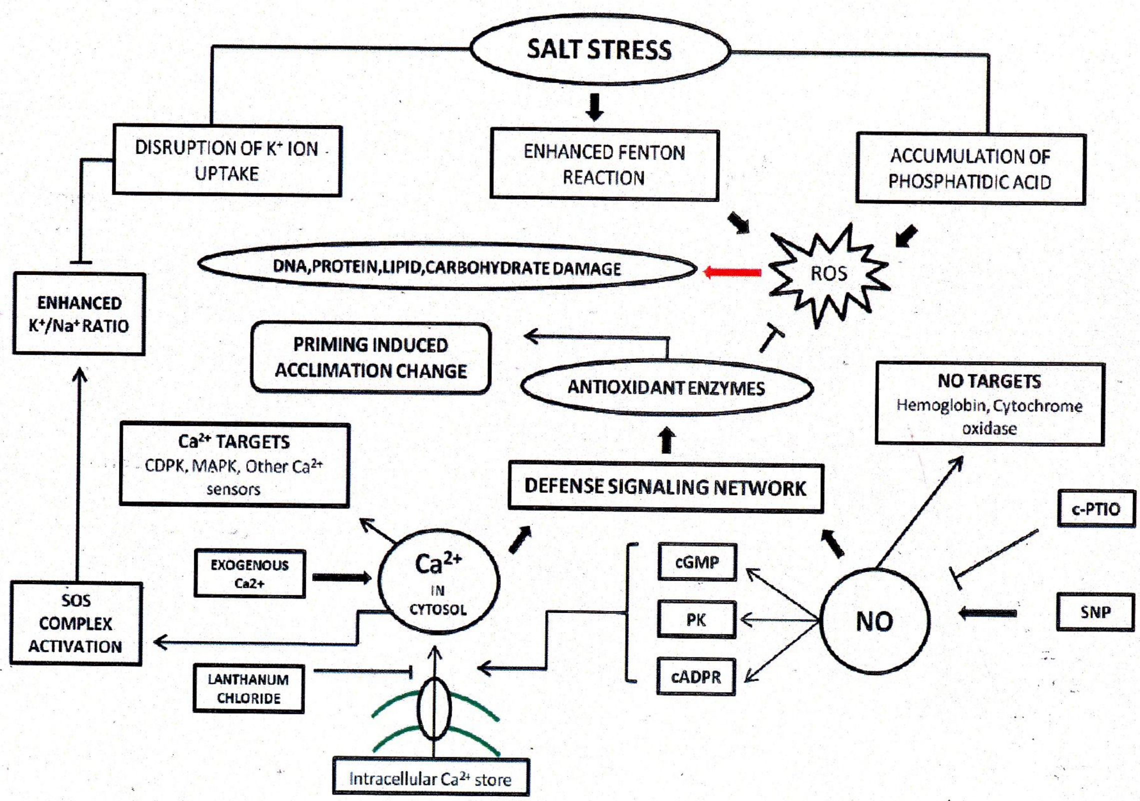

Fig. 4. Interplay of Nitric oxide and Calcium ion and their role in alleviation of oxidative stress mediated damages under salinity stress.

CML, CDPK and CBL proteins have been found to be present only in plant system (Batistic and Kudla, 2009).The specific binding of Ca^{2+} with Calmodulin7 (Cam7) results in direct interaction and regulation, while other calmodulins are likely to mediate gene regulation via interacting with other transcriptional (co)regulators. Metabolic and biosynthetic processes such as brassinosteroid synthesis are important targets of direct Ca^{2+}-dependent modulation (Du and Poovaiah, 2005), but on the other hand Ca^{2+}-dependent phosphorylation and gene regulation provides the major cellular currencies for transduction of specific Ca^{2+} signals into targeted downstream responses (Harper and Harmon, 2005).

Implication of NO for maintenance of redox homeostasis during salinity stress

NO is said to possess considerable capacity to regulate oxidative stress mediated damages along with the level and toxicity of ROS.

The properties of NO which makes it capable to exert a protective function against oxidative stress mediated damages as suggested by Yadav (2010) are given below:

i. It reacts with lipid radicals and stops the propagation of lipid oxidation.
ii. Scavenging the superoxide anion and formation of peroxynitrite which is toxic for plant are later neutralized by ascorbate and glutathione.
iii. Involvement in the activation of antioxidant enzymes.

The lupin seeds when subjected to sodium nitroprusside (SNP) treatment showed better germination under saline stress as well as heavy metal stress (lead and cadmium) suggesting involvement of NO in auxin signalling pathway (Kopyra and Gowdz, 2003). Later, Zheng *et al.* (2009) demonstrated that pre-soaking of wheat with SNP for 20h prior to germination resulted in increased germination rate and radicle weight under 300 mM NaCl. Additionally, decrease in Na^+ concentration but increase in K^+ concentration in the seeds were observed thereby indicating role of NO in maintaining a balance between K^+ and Na^+ during germination under salt stress. The pre-treatment of citrus root with exogenous SNP for a duration of 48h exhibited induction of primary antioxidant responses in the leaves of citrus subjected to salinity stress. The study revealed that SNP pre-treatment enhanced the activity of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) also prevented the NaCl-dependent protein oxidation (Tanou *et al.*, 2009). Zheng *et al.* (2009) claimed in their study that NO treatment effectively contributed to better accumulation of ferritin, a protein active in chelation of excess of ferrous ion present in the cellular system of barley plant subjected to salt stress. Exogenous application of NO through different modes have reported to be effective in regulating the functioning of photosynthetic pigments (Ruan *et al.*, 2002;), improving salt tolerance by modulating proton pump activity in maize (Zhang *et al.*, 2006), regulating osmotic balance and proline metabolism in tobacco (Ke *et al.*, 2013) and prevention of mitochondrial oxidative damage (Zheng *et al.*, 2009). Furthermore, various evidences have been provided by researchers about the protective effect of NO during other stress conditions besides salinity; alleviating the negative effects of UV radiations in wheat seedlings (Yang *et al.*, 2013); mitigating the oxidative injuries under heavy metal stress in lupin seeds (Kopyra and Gowdz, 2003) and modulating the metabolism of biochemicals during osmotic stress (Ke *et al.*, 2013). Other beneficial effects of NO donor reported are regulation of seed germination in *Senna macranthera* (da Silva *et al.*, 2015); maintenance of optimum Na^+/K^+ ratio in cotton seedlings (Dong *et al.*, 2014); enhancement in the enzymes involved in nitrogen metabolism namely nitrate reductase and nitrite reductase in tomato (Manai *et al.*, 2014) also reduction of lipid peroxidation, hydrogen peroxide and superoxide anions; elevation in the activity of major antioxidant enzymes accompanied with increase in the accumulation of biochemicals such as proline, glutathione and sugars under salnity stress in numerous plant system (Hayat *et al.*, 2012; Dong *et al.*, 2014; da Silva *et al.*, 2015;Hameed *et al.*, 2015; Ahmad *et al.*, 2016).

Multifunctional response of calcium in plant system during salinity stress

Calcium is considered as multifunctional element in plants besides as a nutrient, it is involved in several physiological processes like maintenance of membrane integrity, cell wall structure, increasing the activity of key enzymes and phytohormones interaction (Barker and Pilbeam, 2007). Additionally, it plays vital role in signalling network as a secondary messenger under varied environmental conditions (Tuteja, 2009; Batistic and Kudla, 2012). By virtue of this property it is capable of ameliorating the adverse effects of abiotic stresses including chilling, thermal, drought, heavy metals and salinity (Ma *et al.*, 2005; Shao *et al.*, 2008; Siddiqui *et al.*, 2011; Zehra *et al.*, 2012).

Many authors have suggested the beneficial role of calcium ion in the alleviation of the adverse effects of abiotic stress conditions. Therefore, the maintenance of optimum supply of calcium in saline soil is considered as an important factor in preventing the severity of specific ion toxicities, in those crops which are susceptible during salinity stress injury (Grattan and Grieve, 1999). In their study Hasegawa *et al.*, (2000) suggested that during salt stress, plants are able to tolerate high saline concentration by inducing the signal transduction cascades involving calcium ion. Thus, when exposed to stress conditions including salinity plants increase the cytosolic Ca^{2+} accumulation to combat the oxidative damages. Although the basic mechanism involved has remained unexplained, prevailing models for Ca^{2+} functioning include both membrane stabilisation and signalling significance. Considering the potential role of calcium ion in overcoming the negative impacts of several stresses, it has been implemented in various modes in order to provide stress tolerance to plants. Jaleel *et al.*, (2007) demonstrated that when *Catharanthus roseus* plants were supplemented with calcium chloride under drought condition, calcium ion provided osmoprotection to the plants along with increase in glycine betaine accumulation and indole alkaloid content in the shoot and roots of the plant. Also, a significant enhancement in the activity of antioxidant enzymes namely superoxide dismutase, catalase and peroxidase was reported in the same plant subjected to salinity stress (Jaleel *et al.*, 2007). According to Khan *et al.*, (2009) when calcium chloride was applied to linseed in combination with gibberellic acid proved more effective in ameliorating the negative effects of NaCl stress. It was found that the electrolyte leakage of membranes was reduced considerably with decrease in the accumulation of lipid peroxides and hydrogen peroxide. Later Sharma and Dhanda, (2015) suggested the protective role of calcium chloride treatment in *Vigna radiata* in which it was found that the presence of calcium ion helped in maintenance of photosynthetic pigments under salt stress. Similarly, calcium was found to maintain the rate of photosynthesis in *Zoysia japonica* under drought stress by reducing the damage of photosynthetic pigment (Xu *et al.*, 2013); increasing the germination rate and growth of forest strand under simulated acid rain (Liu *et al.*, 2011); involved in the enhancement of chilling stress in *Stylosanthes guianensis* by interacting with abscisic acid (Zhou and Ghou, 2009); the application of calcium in the culture medium was found to activate the accumulation of flavonol in *Polygonum hydropiper* (Nakao *et al.*, 1999); also increase in the activity of antioxidant enzymes, regulation of biochemical metabolism and maintenance of membrane integrity by calcium has been reported in plant system under various stress conditions (Jaleel *et al.*, 2007; Khan *et al.*, 2009; Zhou and Ghou, 2009; Xu *et al.*, 2013; Sharma and Dhanda, 2015).

Crosstalk between Nitric Oxide and Ca^{2+}

The complex cross-talk between NO and Ca^{2+} involve components of Ca^{2+} signalling machinery modulated by NO-dependent mechanisms at post-translational and/or transcriptional levels (Besson-Bard *et al.*, 2008). It is considered that NO regulates the overall control of Ca^{2+} homeostasis by regulating almost all types of associated Ca^{2+} channels and transporters. NO controls the Ca^{2+} homeostasis either via S-nitrosylation of the concerned proteins or through other second messengers namely, cGMP and cyclic ADP ribose (cADPR) (Willmott *et al.*, 1996;

Clementi, 1998 ; Stamler *et al.*, 2001; Ahern *et al.*, 2002; Hess *et al.*, 2005) (Fig. 4).

cADPR is a Ca^{2+} mobilizing messenger that induces release of Ca^{2+} from intracellular Ca^{2+} stores into various plant cells by activating the Ca^{2+} permeable channel ryanodine receptors (Allen *et al.*, 1995; Fliegert *et al.*, 2007). The rise in the cytosolic Ca^{2+} concentrations accompanied with an influx of Ca^{2+} from the extracellular space was observed in *Vicia faba* and *Nicotiana plumbaginifolia* when exposed to NO donors (Garcia-Mata *et al.*, 2003; Lamotte *et al.*, 2004). Previous studies suggested ryanodine receptors -like channels act as a main target of NO action and cADPR as key intracellular messenger responsible for mediating NO signals (Willmott *et al.*, 1996; Clementi, 1998). Interestingly, parallel investigation revealed that exogenous NO was unable to trigger any changes in Ca^{2+} concentration in *Nicotiana plumbaginifolia* cells (Lecourieux *et al.*, 2005). Therefore, these findings indicate that the regulation of Ca^{2+} homeostasis by NO might be restricted to specific cellular compartments.

Furthermore, studies on *Vicia faba* guard cells and *Nicotiana plumbaginifolia* cells revealed that protein kinase inhibitors significantly suppressed the NO mediated elevation of $[Ca^{2+}]$cyt which indicates that besides cADPR involvement of protein kinases is also essential for signalling cascades that relay NO signals to Ca^{2+} channels (Sokolovski *et al.*, 2005; Lamotte *et al.*, 2006).

Interestingly, it has also been suggested that elicitor-induced NO production is enhanced by an upstream influx of extracellular Ca^{2+} (Lamotte *et al.*, 2006; Vandelle *et al.*, 2006). In agreement to above cited literature, a plasma membrane *Arabidopsis* cyclic nucleotide-gated channel (CNGC) member: CNGC2 was identified as a key Ca^{2+} channel which links the rise in Ca^{2+} influx to downstream NOS-like mediated NO production (Ali *et al.*, 2007). Further exploring the complexity of interplay between Ca^{2+} and NO, a study by Vandelle *et al.* 2006 suggested that NO might down regulate its own Ca^{2+}-dependent synthesis by inhibiting elicitor-induced influx of extracellular Ca^{2+}. This negative feedback mechanism is proposed as a strategy to protect the cells from the adverse effects of excessive accumulation of NO as well as Ca^{2+}.

The evidence summarized above documents the complexity of the interaction between NO and Ca^{2+}, but still a substantial effort is required to understand the mechanisms by which NO modulates fluxes of Ca^{2+} and its cellular homeostasis. Another unresolved issue concerns the impact of interplay between these two signalling molecules on the cell response.

Conclusion

Salinity management strategy is very essential for the production with better yield and quality of agricultural crops. Though some group of plants have developed defence mechanism against the salinity stress, but most of the agricultural crops are found to be susceptive. Therefore, several techniques have been suggested by the scientists for overcoming this problem and the implementation of signalling molecules and other metabolites as potent elicitors has showed considerable success in this field. The signalling molecules NO and Ca^{2+} due to their versatile characteristics are reported to be involved in almost all the processes in plant system. Among which their role in providing salinity tolerance to plants could be of great benefit to agricultural sector. From this review it can be suggested that NO and Ca^{2+} are not only stress signalling molecules but they have active role as intrinsic signal in developmental aspects. Furthermore, extensive study on genetics, proteomics and metabolomics along with additional physiological approaches are essential for better understanding of the mechanistic involvement of both NO and Ca^{2+} in the transduction pathways and their interplay among themselves, other factors and their perception and signal transmission to specific downstream responses.

Acknowledgement

The authors gratefully acknowledge Department of Science and Technology, Government of India for the financial assistance provided under the INSPIRE Scheme.

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