

The taxonomy and report of flowering in *Lemna* L. (Lemnaceae) in India

The Lemnaceae members, commonly known by duckweeds, are smallest in size among the angiosperm families. They are found in relatively stagnant aquatic habitats; however, when they occur in running water, they limit themselves to the shores, often among floating leaved species. They grow aggressively and in ideal conditions, capture the whole relatively/partially stagnant habitats within a week. Their taxonomy, mode of propagation and dominance are important in view of their invasive ability in water bodies. Many names have appeared in the literature for its members, but a good number of them lack clarity for proper recognition and identification. The lack of clarity in identity is due to their small size, much reduced plant body (frond), through which only a few features can be defined in the protologues and finally, lack of type as well as authenticated material corresponding to the names. Here we discuss in detail the genus *Lemna*, reporting six species with flowering in four of them. Hooker¹ described six species – *L. minor* L., *L. paucicostata* Hegelm., *L. gibba* L., *L. trisulca* L., *L. polyrrhiza* L. and *L. oligorrhiza* Kurz. *L. paucicostata* is now synonymized under *L. aequinoctialis* Welw. Two of them have been shifted to other genera (*L. polyrrhiza* = *Spirodela polyrrhiza* (L.) Schleid. and *L. oligorrhiza* = *Landoltia punctata* Les & Crawford). Prain² listed four species – *L. paucicostata*, *L. trisulca*, *L. polyrrhiza* and *L. oligorrhiza*. Karthikeyan *et al.*³ enumerated four species – *L. gibba*, *L. trisulca*, *L. minor* and *L. perpusilla*. Cook⁴ described six species – *L. gibba*, *L. trisulca*, *L. minor*, *L. turionifera*, *L. tenera* and *L. aequinoctialis*. *L. tenera* was included based on a collection from Pegu Island, Myanmar. Six species are known to be occurring in India as of now which are keyed out, described and illustrated. The family Lemnaceae in which the genus *Lemna* is tagged is characterized by the following features.

Small aquatic, floating, annuals (perennials cited by some needs correction), with or without roots; plant body reduced to small oval, flat or globose leaf-like frond, purplish or not beneath; pouches two, lateral, sideways, distant from roots.

Daughter plants produced in pouches, with a short stipe or not, similar to mother frond in all respects, including in the presence of primordial roots; often 2–3 generations of baby plants seen one inside another; in some situations, they produce vegetative structures known by turions; turions oval in shape, olive to brown in colour, rootless, surpass overwintering, produce roots once favourable condition appears. Flowers confine to lateral pouches, appear when vegetative buds not present, unisexual, both sexes invariably together; female flowers consist of single pistil, mature first or alternately after the maturity of one of the two male flowers adjacent to it; male flowers consist of 1 or 2 stamens; fruits utricles.

Gray⁵ used an older family name 'Lemnaceae' under which Hegelmaier^{6,7} considered two tribes – Lemneae and Wolffieae. In the later years many authors^{8–12} divided it into two subfamilies – Lemnoideae and Wolffioideae; the distinction essentially lies in the presence/absence of roots.

Subfamily – Lemnoideae Engler in Engler & Prantl. Nat. Pfl. II, 3: 163. 1889.

Fronds variable in shape – round, elliptic, reniform, obovate or lanceolate, with or without pigment cells beneath. Roots present, variable in number, 1–21. Dorsal and ventral scale present (*Spirodela*) or indistinct (*Landoltia*) or absent (*Lemna*). Stipe present, hyaline, often prominent (*Spirodela*) or not (*Landoltia* and *Lemna*). Inflorescence lateral, consisting of two male and one female flowers; filaments prominent when flowers mature; anthers bilocular; dehiscence transverse (*Lemna*) or longitudinal (*Spirodela* and *Landoltia*).

The three genera – *Spirodela*, *Landoltia* and *Lemna* recognized in this subfamily can be distinguished as below.

Key to genera

1a. Root 1; root tracheids absent; dorsal and ventral scales absent; anther dehiscence transverse *Lemna*
1b. Roots more than one; root tracheids present; dorsal and ventral scales present; anther dehiscence longitudinal Group 2

2a. Roots two, root tracheids confining to base only; frond oblong–elliptic; dorsal and ventral scale absent; venation obscurely three-nerved *Landoltia*
2b. Roots 4–21; root tracheids extending up to tip; frond round or ovate; prominent dorsal and ventral scale present; venation distinctly 3–7-nerved *Spirodela*.

Lemna L., Sp. Pl. 1: 970. 1753. Type – *Lemna minor* L.

Lenticularia Ség., Pl. Veron. 3: 129. 1754. *Lenticula* Hill., Brit. Herb.: 530. 1756. *Hydrophace* Haller, Hist. Stirp. Helv. 3: 68. 1768. *Telmatophace* Schleid., Linnaea 13: 391. 1839.

Fronds solitary or in groups; symmetric or asymmetric; elliptic, oblong or lanceolate; flat or gibbous, often with 1–3 dorsal papillae on the median line of the frond. Root one. Inflorescence with two male and one female flowers in lateral budding pouches. Male flowers two; anthers dehisce transversely. Female flower one. Fruit utricle.

About 13 species (in five sections) distributed all over the world. Six of them, from three sections, are reported from India. Flowers photographed using Nikon SMZ 1500 camera at different magnifications.

Key to the sections

1a. Margin of the fronds entire at base, denticulate above; base of the frond forming a distinctly and long green stalk sect. Hydrophylla
1b. Margin of the fronds entire throughout; green stalk absent Group 2
2a. Root sheath winged at base; root tips sharply pointed sect. Alatae
2b. Root sheath not winged at base; root tips rounded sect. Lemna.

Section Hydrophylla Dumort (type: *L. trisulca* L.)

1. *Lemna trisulca* L., Sp. Pl. 2: 970. 1753. Type: Lecto: LINN, photo! (Howard, 1979). *Staurogeton trisulcus* (L.) Schur, Verh. Mitth. Siebenburg. Vereins Naturwiss. Hermannstadt 4: 70. 1853. *Hydrophace trisulca* (L.) Bubani, Fl. Pyren. 4: 34. 1897. *Lenticula trisulca* (L.) Scop., Fl. Carniol. 2: 213. 1772. *Lenticula ramose* Lam., Fl. Franç. 2: 189. 1778. *Lemna cruciata* Roxb., Fl. Ind. 3:

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1832. *Lemna intermedia* Ruthe, Fl. Mark Brandenburg 2: 277. 1834. *Lemna bisulca* Veesenm., Beitr. Fl. Russ. Reich. 9: 104. 1854. *Lemna trisulca* var. *pygmaea* Henn., Verh. Bot. Vereins Prov. Brandenburg 33: 8. 1891 (Figure 1 a).

Fronds dark green, 3–50 together, narrowly ovate, 11 × 2 mm, relatively long in comparison to other species, thinner or flat (in herbarium specimens), submerged just beneath water surface, and not float-

ing, 3–50 attached together through prominent stipes forming a branched chain/colony; stipes very long 4–10 mm; lamina narrowed at base forming a long, green stalk; margins entire at base, denticulate near the apex; nerves three, indistinct. Roots one, 3–9 mm often not developed; sheath absent; root cap pointed. Flowering and fruiting not seen.

Distribution in India: Manipur, Odisha and West Bengal.

Section *Lemna* (Type: *L. minor* L.)

The section comprises of about seven species, three of them occur in India.

Key to the species

- 1a. Fronds produce turions; papulae indistinct and multiple in a line in median region *L. turionifera*
- 1b. Fronds never produce turions; papulae absent or when present only 1 at tip Group 2
- 2a. Fronds often gibbous, papillae absent *L. gibba*
- 2b. Fronds never gibbous; papillae present *L. minor*.

2. *Lemna gibba* L., Sp. Pl. 2: 970. 1753. Type: Lecto, BM! (Hashimi & Omer, 1986). *Lenticula gibba* (L.) Moench, Methodus 319. 1794. *Telmatophace gibba* (L.) Schleid., Linnaea 13: 391. 1839. *Lenticula gibbosa* P. Renault, Fl. Orne.: 40. 1803. *Lemna cordata* Sessé & Moc., Pl. Nov. Hisp. 159. 1890. *Lemna parodiana* Giardelli, Notas Mus. La Plata. Bot. 2(12): 97. 1937 (Figures 1 b and 2 a).

Fronds 3–4 together hidden/apparent in a chain in lateral pouches, representing 3–4 consecutive generations, light green, ovate to oblanceolate, 5–8 × 0.4–5 mm; upper surface shining; papulae absent; nerves 3–4 originating from the node, but extremely faint. Roots one, 3–4 mm long; root cap mostly rounded, 0.4–0.8 mm; stipe absent. Inflorescence in lateral pouch; male and female flowers together, male flowers two; stamens ca. 0.2 mm; anther bilocular, ca. 0.1 mm dia.; female flowers one, gynoecium ca. 0.3 mm.

Distribution in India: Gujarat, Jammu and Kashmir, Punjab and West Bengal.

Note: It is a very characteristic species in this section because of its gibbosity. But sometimes flat fronds are produced which are more or less identical to *L. minor* and the absence of apical papulae bears a clue in its recognition.

3. *Lemna minor* L., Sp. Pl. 2: 970. 1753. Type: Lecto, LINN, photo!, Herb. Linn. No. 1093.2 (Hepper, 1973). *Lenticula minor* (L.) Scop., Fl. Carniol. 2: 213. 1772. *Hydrophace minor* (L.) Bubani, Fl. Pyren. 4: 23. 1897. *Lenticularia vulgaris* Lam., Fl. Franç. 2: 189. 1778. *Lemna minima* Thuill. ex P. Beauv., J. Phys. Chim. Hist. Nat. Arts 82: 113. 1816. *Lemna minor* var. *oxymitra* Hegelm., Lemnac. 143. 1868. *Lenticularia monorhiza* Montandon, Guide Bot.: 308. 1868. *Lemna rwandensis* De Sloover, Bull.

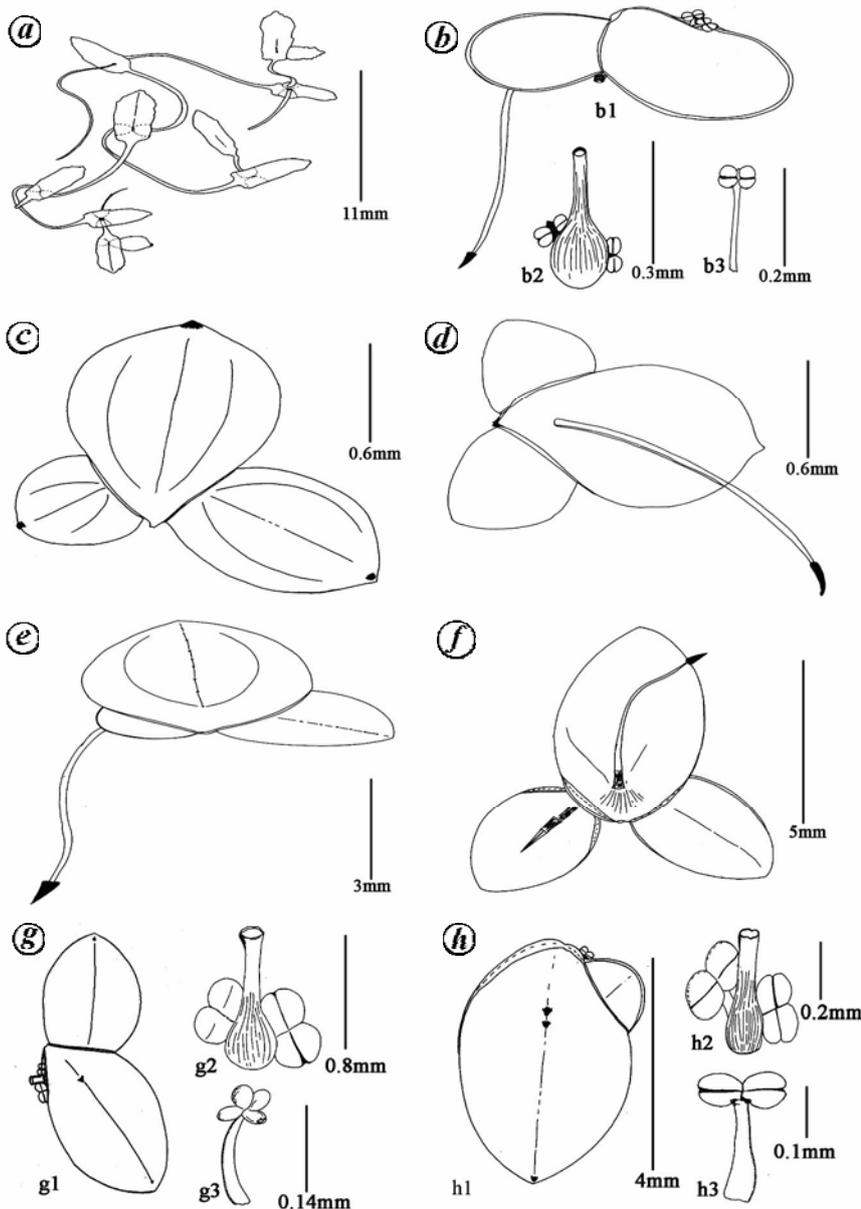


Figure 1. a, *Lemna trisulca*. b, *L. gibba* – b1, Flowering frond; b2, Mature female flower with two male flowers; b3, Mature male flower. c, d, *L. minor*, dorsal and ventral view of the frond. e, *L. turionifera* dorsal view of frond. f, g, *L. aequinoctialis* – f, Ventral view of frond. g1, Flowering frond; g2, Mature female flower with two male flowers; g3, Mature male flower. h, *L. perpusilla* – h1, Flowering frond; h2, Mature female flower with two male flowers; h3, Mature male flower.

Jard. Bot. Belg. 43: 366. 1973 (Figures 1 c, d and 2 b).

Fronds dark green, 5–10 together, ovate, 1–8 × 0.5–5 mm, flattened. Upper surface with distinct papillae near apical region; nerves 3–4, not reaching tip, distinct. Stipe small, 0.1–0.2 mm hyaline. Roots one, ca. 12 mm; root cap rounded; wing absent. Inflorescence on lateral pouches, male and female flowers together. Male flowers two, ca. 0.3 mm; anther bilocular with transverse dehiscence line, ca. 0.2 mm dia. Female flow-

ers one, ca. 0.15–0.2 mm. Fruiting not observed.

Distribution in India: almost throughout India.

4. *Lemna turionifera* Landolt, Aquatic Botany: 355. 1975. Type: Holo. ZT (Landolt, 1986; Figure 1 e). Fronds dark green, usually 2–3 together, nearly orbicular in shape, 12 × 0.9 mm, flattened; papillae relatively small, 4–5, along dorsal median line; nerves three, distinct, not reaching tip. Roots one, not winged; root cap acute; budding pouches lateral,

turions brown, often produced in lateral pouches. Flowering and fruiting not known.

Distribution in India: Jammu and Kashmir, Himachal Pradesh and West Bengal.

Note: Overwintering turion makes this species interesting and important. It flowers occasionally¹³. But in the present study the authors could not collect the flowers.

Section Alatae Hegelm. (Type: *L. perpusilla* Torrey)

This section contain two species – *L. perpusilla* and *L. aequinoctialis*, which are closely related and difficult to separate if not critically examined.

Key to the species

- 1a. Fronds bear 2–3 larger papillae above the node and one smaller near the tip ... *L. perpusilla*
- 1b. Fronds bear one small papulae above the node and one larger near the tip ... *L. aequinoctialis*.

5. *Lemna perpusilla* Torr., Fl. New York 2: 245. 1843. Type: Lecto, photo! STU; Isolecto, GH (00061635), MO!, KANU, NY (Landolt, 1986). *Lemna perpusilla* var. *trinervis* Austin ex Gray, Manual ed. 5: 479. 1867. *Hydrophace perpusilla* (Torr.) Lunell, Amer. Midl. Naturalist 4(6): 237. 1915 (Figures 1 h and 2 f–h).

Fronds dark green, solitary or 2–3 together, usually one visible through pouch, orbicular-ovate 3–4 × 0.8–2 mm long; upper surface often with 2–3 papules above the node which are bigger than the apical one. Stipe absent. Roots one; root sheath winged, 0.2–0.5 mm. Inflorescence produced in lateral budding pouches; male flowers 2, ca. 0.14 mm; anthers divaricate, globular, ca. 0.01 mm, dehisce by transverse slit. Female flower composed of one gynoecium, ca. 0.8 mm; ovary globose, hyaline, with 2–3 ovules; style one, terminal, short; stigma one, cup shaped, produces stigmatic fluid during pollination. Fruit utricle, ca. 0.05 mm, attached on the inner surface of the pouch.

Distribution in India: throughout the country.

Note: Landolt¹³ has stated that this species does not occur in India based on his study of herbarium specimens only. The present authors have collected it from West Bengal and Bihar.

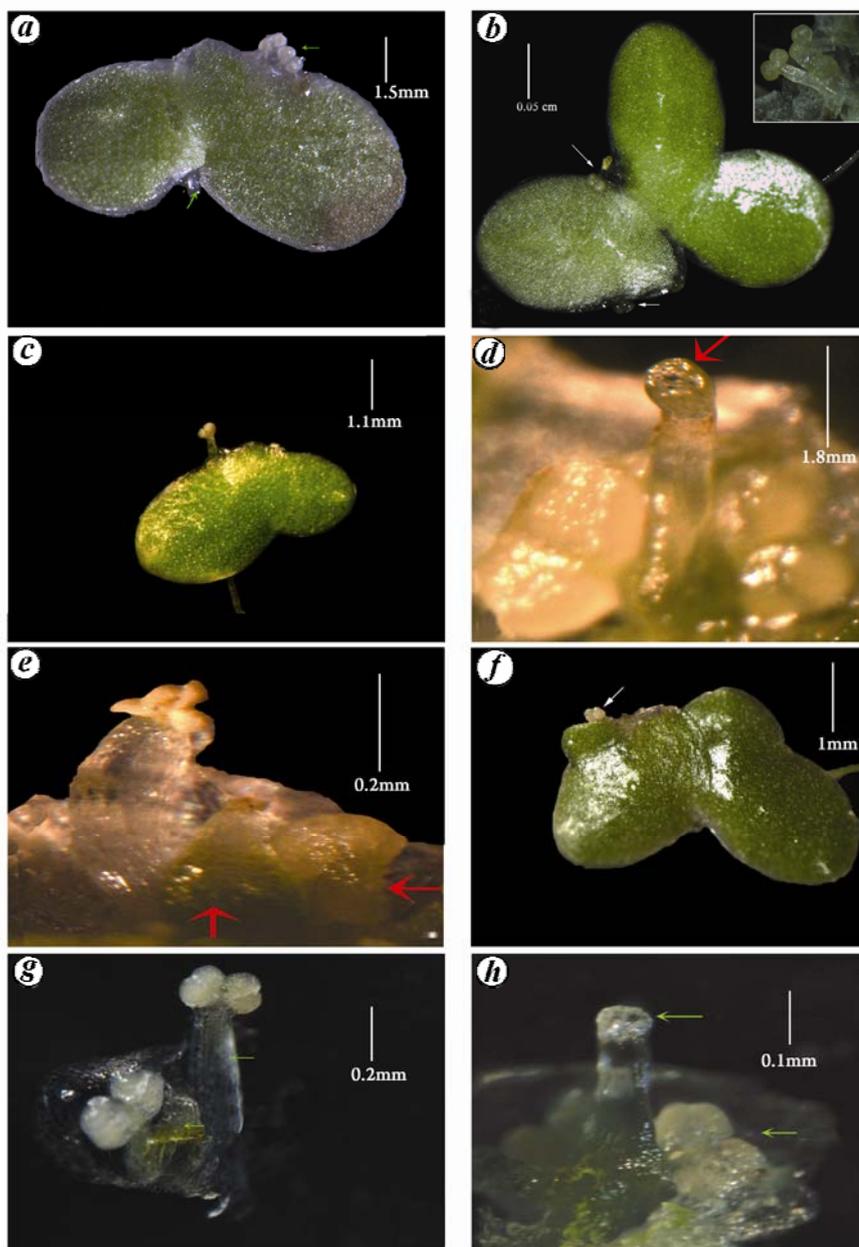


Figure 2. a, *Lemna gibba* flowering frond. b, *L. minor* – flowering frond with flowers (inset). c–e, *L. aequinoctialis* – c, Flowering frond. d, Mature female flower with two male flowers; e, Dehiscing anther. f–h, *L. perpusilla* – f, Flowering frond; g, Two mature male flowers with female flower after fertilization and h, Mature female flower with two immature male flowers.

6. *Lemna aequinoctialis* Welw. Apont.: 578. 1859. Type: Angola; Prov. Luanda, Distr. Luanda; 1858, F. Welwitsch 206. Lecto, photo! STU; Isolecto, BM, G, K, ZT. (Landolt, 1986). *Lemna angolensis* Welw. ex Hegelm., J. Bot. 3: 112. 1865. *Lemna paucicostata* Hegelm., Lemnac. 139. t. 8. 1868. *Lemna paucicostata* var. *membranacea* Hegelm., Lemnac.: 141. 1868. *Lemna trinervis* (Austin ex Gray) Small, Fl. S.E.U.S. 230. 1903 pp. *Lemna minima* Blatt. & Hallb., J. Indian Bot. 2: 50. 1921. *Lemna blatteri* McCann, J. Bombay Nat. Hist. Soc. 43: 153. 1942. *Lemna aoukikusa* T. Beppu & Murata, Acta Phytotax. Geobot. 36: 55. 1985 (Figures 1 b, g and 2 c-e).

Fronds light green, usually 1–3 together, oblong or ovate or orbicular, 2–5 × 0.13–0.09 mm, asymmetrical; two distinct papulae on the dorsal surface; nodal (where the veins converge) papillae smaller than apical one. Roots one; root sheath winged, ca. 0.01 × 0.02 mm. Inflorescence on two lateral pouches. Male flowers two, ca. 0.1 mm in length; anthers divaricate, bilocular, dehisce by transverse slit. Female flower composed of gynoeceium; ca. 0.2 mm long; ovary globose, hyaline; style one, terminal. Fruit utricle.

Distribution in India: throughout the country.

Note: Though isolecto is said to be in BM (The Natural History Museum, London) and ZT (Edgenossische Technische Hochschule Zurich, Switzerland), the concerned databases of these herbaria do not show these types. But these types are included based on Landolt (l.c.).

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ACKNOWLEDGEMENTS. We thank the curators of BM (for *L. gibba*, *L. minor* and *L. trisulca*) and STU (for *L. perpusilla* and *L. aequinoctialis*) for images of the types; Prof. E. Landolt (ZT) for confirmation of identity of different collections, and the Director, BSI, Kolkata for facilities and fellowship to S.H. under the Flora of India programme.

Received 29 August 2011; revised accepted 15 May 2012

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Bt Cry toxin expression profile in selected Pakistani cotton genotypes

Pakistan is ranked fourth among the top five cotton-producing countries in the world. About 70–80% of the cultivated area is under *Bt* cotton in Pakistan¹. Bollworm (*Helicoverpa armigera*) is the major pest of cotton². It causes 31.73–36.45% yield losses^{3,4} and these are reduced by heavy pesticide application. There has been a tremendous increase in the import and use of pesticides. Consequently, about 7.7 billion rupees is spent on pesticides every year⁵. Considering the total pesticide usage (94,265 metric tonnes in 2007–08), 70% is being used exclusively on cotton. In addition to being a pollutant, pesticides are also hazardous to the farmers and livestock¹. The large amount of money being spent on these chemicals can be avoided by planting *Bt* cotton.

From biosafety point of view, *Bt* biopesticides are better than chemical pesti-

cides. Because the *Bt* toxins are highly specialized and have no negative effect on the environment⁶. The effectiveness of *Bt* cotton depends on the expression of insecticidal genes⁷ and does not remain constant throughout the growing season⁸. The performance of *Bt* genes for controlling target insect pests varies according to the cotton varieties⁹, age of the plant¹⁰, different parts of the plant¹¹, types of gene and also the insertion sites of the gene into the DNA of target plants^{12–14}.

In Pakistan, the shift to *Bt* cotton was slow due to non-existence of necessary infrastructure. Plant breeders developed *Bt* varieties using local genotypes through backcrossing with alien *Bt* cotton varieties having the *CryIAC* gene of non-patented event (MON531) in Pakistan. In 2010, approximately 600,000 farmers cultivated *Bt* transgenic cotton varieties¹⁵. A study estimated that 81%

and 90% were confirmed *Bt* varieties having only the *CryIAC* gene, in Sindh and Punjab provinces respectively¹. The introduction of Bollgard-II event is expected soon as negotiations between the Government of Pakistan and Monsanto, USA are in progress.

To reduce the risk of resistance development in target insect pests against *Bt* cotton, there is a need to understand the variations in efficiency of *Bt* genes and their mechanisms. For this, advanced bioassay techniques like ELISA have been used to measure the quantity variations in the *Bt* Cry (crystal) proteins¹⁶.

The main objective of the present study was to quantify actual *Bt* toxin levels in cotton genotypes at the growth stages when they are attacked by bollworm. The trend was also studied in plant parts (i.e. leaves and squares)