Tospoviruses are emerging as a major constraint in cultivation of various leguminous, solanaceous and cucurbitaceous crops in the Indian subcontinent. Watermelon bud necrosis virus (WBNV) is one of the important viral pathogens of watermelon (*Citrullus lanatus*) causing bud necrosis which results in 100% yield losses in some areas. The complete genome information of WBNV has not been reported and therefore WBNV is considered as a tentative species under the genus, *Tospovirus*. Cultivars resistant to WBNV are not known and other management options are also not suitable for improving resistance in watermelon against WBNV. Transgenic cultivars resistant to virus have been developed for other cucurbits. However transgenic watermelon plants resistant to WBNV are not available.

Watermelon plants showing yellowing and bud necrosis symptoms were collected from the experimental fields of Indian Agricultural Research Institute (IARI), New Delhi during May, 2008. In the present study, the complete sequence of M RNA of a watermelon isolate of WBNV originating from northern India, optimization of *in vitro* plants regeneration protocols using different explants, development of transgene constructs with nucleocapsid (NP) gene of WBNV and transgenic watermelon cv. Sugar Baby plants with *GUS* and *WBNV*-NP genes have been achieved. The findings of present investigation are as follows:

- The Watermelon bud necrosis virus Delhi isolate from watermelon host (*WBNV*-wDel) was established in the greenhouse on *Nicotiana benthamiana* and *Vigna unguiculata* through sap-inoculation. Association of WBNV with the field and sap-transmitted samples was identified by enzyme-linked immunosorbent assay using polyclonal antisera to Groundnut bud necrosis virus (GBNV) and *Watermelon silver mottle virus* (WSMoV) and polymerase chain reaction (PCR) using primers to N gene of WBNV (Jain *et al*., 2005).

- Natural infection of WBNV was observed in Watermelon at Delhi (IARI Experimental fields, Yamuna bank, Majnutila and ISBT), Haryana (Balabhgarh and Gurgaon) and Uttar Pradesh (Kanpur and Lucknow). The disease incidence was about 20-60% in Uttar Pradesh and Haryana and 50-70% in on the bank of Yamuna river in New Delhi. While in experimental fields of IARI, New Delhi, the disease incidence was about 90-100% which resulted in death of the plants within a week.

- The host range of WBNV-wDel isolate was ascertained. The virus was transmitted to members of Solanaceae, Fabaceae, and Cucurbitaceae.
Symptomatology of WBNV and GBNV produced similar symptoms (chlorotic lesion or rings, mottling and rapid wilting) on *N. benthamiana*, however they could be differentiated in cowpea cvs Pusa Komal and C-152, peanut and watermelon. WBNV could not be sap transmitted to peanut and GBNV to watermelon.

WBNV produced local chlorotic lesions and ring spots on Pusa Komal and C-152, respectively, whereas GBNV produced chlorotic lesions followed by necrotic lesions in cowpea. Further, only GBNV produced systemic necrosis in leaves and stems of cowpea.

The complete nucleotide sequence the M RNA segments of WBNV-wDel isolates was determined, and compared with WBNV-som isolate and other tospoviruses.

The complete M RNA of WBNV consisted of 4794 nt, composed of 31.6% A, 33.2% U, 17.8% C and 17.3% G.

The genome contained two non-overlapping open reading frames (ORFs) in ambisense orientation separated by 402 nt A-U-rich intergenic region (IGR).

The first ORF towards 5´ end was 924 nt long in the viral (v) strand that started with an ATG codon at 56 nt and ended at 979 nt with a UAA codon. The ORF encoded a protein of 307 amino acids with a predicted molecular mass of 34.22 kDa and was similar to NSm gene of the other tospoviruses.

The second was ORF was larger and located towards 3´ end containing 3366 nt in viral complementary strand of the genome that started with ATG at 4747 nt and ending with a stop codon, UAA at 1382 nt (numbered from 5´ end of the vRNA). Based on similarities with the other tospoviruses, the larger ORF encoded the precursor of the glycoproteins, Gn/Gc containing 1121 amino acids with a predicted molecular mass of 127.15 kDa.

The 5´ and 3´ untranslated regions (UTR) of the M RNA were 55 and 47 nt long, respectively forming a panhandle consisting of 22 nt having 19 complementary pairs and three mismatches at the terminal ends.

The M RNA genome sequence of WBNV-wDel was compared with that of WBNV-Wm-Som isolates and nine different tospoviruses. Wm-Som isolate was one nt longer than wDel isolate sharing 91.6% sequence identity.

The 5´ UTR and NSm of Wm-Som isolate were near identical to wDel isolates; interestingly, Gn/Gc and 3´ UTR were significantly different sharing only 74.6% and 80.8% identity, respectively.
Phylogenetic analysis based on complete mRNA sequence revealed three distinct groups of relatives of WBNV: (i) the close relatives WSMoV serogroup sharing 73.9-79.1% sequence identity; (ii) the intermediate relatives, Iris Yellow spot sero-group and Tomato zonate spot virus (TZSV) sharing 62.0-65.3% sequence identity and (iii) the distant relatives, Impatiens necrotic virus (INSV) and TSWV sharing only 46.3% and 44.7% sequence identity, respectively.

GBNV was closest to WBNV sharing 79.1% sequence identity.

The melon infecting tospoviruses, MYSV and WSMoV known to occur in Japan and Taiwan shared only 63.3% and 75.2% identity, respectively with WBNV.

An efficient and reproducible in vitro regeneration from cotyledon explants have been developed in watermelon cv. Sugar Baby. The factors affecting in vitro regeneration of multiple shoots were optimized. The type and concentration of hormones influenced the frequency of shoot induction and the number of shoots per explant. Explants cultured on MS basal medium produced no callus whereas those cultured on medium containing 6-benzylaminopurine (2.0 mg/l) and indole acetic acid (0.1 mg/l) developed callus which on medium containing 2.0 mg/l BAP and 0.3 mg/l IAA differentiated into shoots. This indicates that cytokinin and auxin are more effective for callusing and shoot induction (76%) from cotyledon explants excised from in vitro raised seedlings.

The explants, epicotyl and hypocotyl excised from seedlings raised in sand developed shoots on MS basal medium supplemented with BAP (3.0 mg/l). However, epicotyl (28%) explants were more responsive for shoot regeneration than hypocotyl explants (18%). The basal halves of cotyledons could not regenerate on the medium containing BAP alone.

The shoots regenerated from cotyledon explants were rooted on MS medium. Shoots with well-developed roots (plantlets) were established in sand where 90% of them survived and developed into morphologically normal plants which subsequently produced flowers and fruits with viable seeds.

Optimization of a threshold concentration of an efficient selection agent kanamycin is very important parameter for transformation system. Kanamycin at 100 mg/l inhibited the shoot regeneration from untransformed (control) explants. Therefore, this concentration was chosen for the selection of transformed shoots.
Various factors affecting transformation efficiency were optimized. Bacterial concentration at $10^7$ cell/ml (0.6 OD at 600 nm), bacterial inoculation for 30 minutes and co-cultivation for 2 days were found to be the optimal. Co-cultivation in the presence of acetosyngone (200 µM) was effective in improving the transformation frequency.

Stable transformation and expression of transgene in watermelon cv. Sugar Baby were achieved using Agrobacterium-mediated genetic transformation system. In-vitro germinated four-d-old cotyledon explants excised from seedlings raised on ½ MS were cocultured with Agrobacterium tumefaciens strain EHA105 carrying a binary vector pBI121, which contained a β-glucuronidase gene and a neomycin phosphotransferase gene (npII), both driven by CaMV 35S promoter. The inoculated explants were cultured on selection medium (MS + 3.0 mg/l BA + 0.3 mg/l IAA + 100 mg/l kanamycin + 250 mg/l augmentin) for the selection of transformed calli and shoots. The shoots recovered on selection medium were subjected to the second round of selection at rooting stage (in presence of kanamycin(10 mg/l)) to eliminate escapants. The putative transformants were successfully analysed for the presence of transgenes by PCR. Stable GUS expression was detected in calli, shoot and leaves. The transformation frequency was 7.8%.

The developed transformation protocol was used to transfer WBNV NP gene to impart resistance to watermelon against WBNV. The presence of transgenes in putative explants was confirmed by PCR and Southern analysis (PCR). The transformation frequency was 0.9%.

In conclusion, the present investigation describes for the first time, the M RNA genome of Watermelon bud necrosis virus (WBNV) isolate from north India. The sequence analysis suggests phylogenetic incongruence in WBNV isolates and confirms further that WBNV is a distinct species under the genus Tospovirus, family Bunyaviridae infecting cucurbits in India. The transgene constructs using full and truncated nucleocapsid protein genes of WBNV were developed. An efficient and reproducible regeneration protocol for watermelon cv. Sugar Baby was optimized and used for Agrobacterium mediated transformation using two different gene constructs carrying a reporter gene (GUS) and WBNV-NP gene which resulted in transformation frequency of 7.8% and 0.9%, respectively. Very low transformation frequency in watermelon Sugar Baby was achieved with viral gene construct. Further work is required not only to improve
the transformation frequency but also to check the resistance of transgenic plants of watermelon to WBNV.