13. Khanna, M., Kumar, P., Choudhary, K., Kumar, B. and Vijayan, V. K., Emerging influenza virus: a global threat. *J. Biosci.*, 2008, **34**, 475–482.
14. Koehler, A. V., Pearce, J. M., Flint, P. L., Franson, J. C. and Ip, H. S., Genetic evidence of intercontinental movement of avian influenza in a migratory bird: the northern pintail (*Anas acuta*). *Mol. Ecol.*, 2008, **17**, 4754–4762.
15. WHO, Manual on Animal influenza diagnosis and surveillance, WHO/CDS/CSR/NCS, 2002, p. 5.
16. FAO, Animal production and health manual, wild birds and avian influenza; an introduction to applied field research and disease sampling techniques, 2007.
17. Krys Kazmierczak, *A Field Guide to the Birds of India, Sri Lanka, Pakistan, Nepal, Bhutan, Bangladesh and Maldives*, Om Book Service, New Delhi, 2000.
18. Pande, S., Tambe, S., Francis, C. F. and Sant, N., *Birds of Western Ghats, Kokan and Malabar* (*including Birds of Goa*), Oxford University Press, Mumbai, 2003.
19. Weber, T. P. and Stilianakis, N. I., Ecologic immunology of avian influenza (H5N1) in migratory birds. *Emerg. Infect. Dis.*, 2007, **13**, 1139–1143.
20. Feare, C. J. and Yasué, M., Asymptomatic infection with highly pathogenic avian influenza H5N1 in wild birds: how sound is the evidence? *Virol. J.*, 2006, **3**, 96.
21. Chen, H. *et al.*, Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. *Proc. Natl. Acad. Sci. USA*, 2006, **103**, 2845–2850.
22. Lvov, D. K. *et al.*, Isolation of influenza A/H5N1 virus strains from poultry and wild birds in west Siberia during epizooty (July 2005) and their depositing to the state collection of viruses (August 2005). *Vopr. Virusol.*, 2006, **51**, 11–14.
23. Chen, H. X. *et al.*, Seroprevalance and identification of influenza A virus infection from migratory wild waterfowl in China (2004–2005). *J. Vet. Med. B Infect. Dis. Vet. Pub. Health*, 2006, **53**, 166–170.
24. Krauss, S., Influenza in migratory birds and evidence of limited intercontinental virus exchange. *PLoS Pathog.*, 2007, **3**, e167.
25. Lebarbenchon, C., Absence of detection of highly pathogenic H5N1 in migratory waterfowl in southern France in 2005–2006. *Infect. Genet. Evol.*, 2007, **7**, 604–608.
26. Winker, K. *et al.*, Movements of birds and avian influenza from Asia into Alaska. *Emerg. Infect. Dis.*, 2007, **13**, 547–552.
27. Rassmusen, P. C. and Anderton, J. C., *Birds of South Asia. The Ripley Guide*, Smithsonian Institution, Washington DC, 2005, vols 1 and 2.
28. Stallknecht, D. E. and Shane, M. E., Host range of influenza virus in free-living birds. *Vet. Res. Commun.*, 1988, **12**, 125–141.
29. Olsen, B., Munster, V. J., Wallensten, A., Waldenstorm, J., Osterhaus, A. D. M. E. and Fouchier, R. A. M., Supporting online material for global patterns of influenza A virus in wild birds, 2006; http://www.sciencemag.org/cgi/content/full/312/5772/384/DCI
30. Alexander, D. J., A review of avian influenza in different bird species. *Vet. Micro.*, 2000, **74**, 3–13.
31. Rao, A. S. R. S., Location of the epicenter of avian bird flu might determine the rapidity of its spread in India. *Curr. Sci.*, 2008, **95**, 314–315.

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Harmonizing soil organic carbon estimates in historical and current data

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Estimation of soil organic carbon (SOC) is indispensable in studies involving soils and global climate change. SOC retention in soil is a function of climate, vegetation and intrinsic soil properties. Historically, SOC estimates are based on wet digestion which gives low carbon recovery. This results in underestimation of its density and stock, however, most of the existing historical and current SOC data sets are based on wet digestion. Hence, we have compared the wet digestion with precise oxidative combustion method for SOC estimation, to develop factors for conversion of historical data into comparable values. It was found that the recovery percentage of SOC is lower than oxidative method and it further decreased with increase in clay content. In case of land use, the recovery percentage is higher in forest soils, followed by agricultural soils and the least in wasteland. A general correction factor of 1.42 and clay content specific correction factors of 1.35, 1.45 and 1.81 are recommended to convert historical data into current reliable SOC estimates.

Keywords: Clay, land use, oxidative combustion, soil organic carbon, Walkley and Black method.

INTEREST in soil organic carbon (SOC) has greatly increased in recent years because terrestrial organic carbon (OC) can be a key factor in understanding the effect of carbon (C) emission on global climate change. The increase of CO_2 from anthropogenic sources has especially been the focus of public concern. Emission of CO_2 from oxidation of soil organic matter or from respiration of the above-ground biomass is one of the largest sources of CO_2 in the atmosphere[1,2]. Researchers are interested in knowing the factors influencing soil as a source or a sink of atmospheric CO_2, apart from SOC content which is considered to be the key soil quality indicator.

Much of the current database on terrestrial C content has been gathered primarily from soil surveys, and the C content were commonly determined by wet digestion method[3–5]. Moreover, many researchers are interested not only in the total soil C content but also in specific components of soil C and the dynamics of its turnover. There could be methodological differences with the change of the analytical procedures and the instruments[6], leading to

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inaccurate and tentative SOC stock assessment and there is a need of finding a soil-specific correction factor.

Over the past 50 years or so, a range of analytical techniques have been used to estimate SOC. The most widely reported procedure is wet digestion by Walkley and Black technique[7] (WB) which utilizes the heat of reaction from the addition of concentrated sulphuric acid to oxidize the SOC by hot chromic acid. But, this heat is inadequate to drive the reaction to completion resulting in SOC recovery of only 75–80%. This recovery equates to a factor of 1.32, to convert the wet digestion values obtained to values equivalent to laborious combustion methods. However, the correction factor is found to vary with the group of soils and is reported to be 1.00 for tropical soils[8,9], 1.40 for colder climate soils[10], 1.63 for Russian chernozem[11]. A recovery of lower than 15% SOC under pasture than under ploughing was observed by Diaz[12]. Lower recoveries of SOC can also be due to local factors such as presence of large amount of charred organic matter from fires[13]. Further, wet digestion requires the use of toxic chromium compounds as oxidants. Hence, the present study was carried out with the objective of a comparative evaluation of wet digestion method (WB) and an oxidative combustion-infrared analysis method using total organic carbon (TOC) analyser for determination of SOC and the effect of clay content and land use on its estimation.

A total of 60 soil samples representing different horizons of 25 soil profiles collected from different land uses (agriculture, forest and wastelands) were selected. These profile samples were collected from six districts (Dewas, Hoshangabad, Indore, Raisen, Sehore and Ujjain) of Madhya Pradesh representing different physiographic units. Prior to analysis, the soil samples were air-dried, ground, and sieved to <0.2 mm diameter. SOC content of samples was determined both by WB method (wet digestion) and dry oxidative combustion (Shimadzu-make TOC analyser) with a solid sample module (SSM-5000A). In dry digestion, the SOC was determined by difference method, where difference of total carbon (TC) and inorganic carbon (IC) was taken. The samples are heated to 900°C in the presence of oxidation catalyst, and the evolved CO_2 is carried by synthetic air to the non-dispersive infrared (NDIR) gas analyser for detection of TC. For IC measurement, the sample is acidified with a small amount of orthophosphoric acid (85%) and heated to 200°C, and the evolved CO_2 is detected by NDIR. The calibration curves that mathematically express the relationship between peak area and TC/IC concentration were generated by analysing various concentrations of TC/IC standard solutions. Potassium hydrogen pthalate and sodium carbonate (Na_2CO_3) were used to prepare standard solutions for TC and IC respectively. These calibration curves gave a maximum error of 0.67% for TC and 0.51% for IC. Hydrometer method was used for determining clay content in soil samples[14].

A summary of SOC results obtained from WB method (after applying standard correction factor of 1.32) and TOC analyser is given in Table 1. The SOC of the study area as estimated by WB method varies from 0.23 to 2.0%, whereas it varies from 0.47 to 2.80% by TOC analyser, relatively higher than WB values. The origin of this difference as indicated earlier is mainly due to low carbon recovery in WB method. A plot of percentage of SOC from WB vs TOC analyser shows R^2 of 0.81 with a slope of 1.42 (Figure 1). It indicated that despite applying a common correction factor of 1.32, WB method underestimates the SOC. This underestimation is taken as percentage of difference in the SOC estimates between the two methods considering TOC analyser based results as reference. The underestimation varies from 12.17 to 66.54% with an average of about 33%. These variable recovery rates were related to laboratory and field conditions and to the sample composition[4]. As a result of this, the total stock and density estimates are reported lower than the actual values which greatly reduces its reliability.

The results of textural analysis indicated that the clay content of the study area varies from 30 to 78% and it mainly contains 2 : 1 type of clay minerals (expanding type). As clay is known to form complex with organic materials apart from physical protection, it reduces the oxidation of organic materials. Hussain and Olson[15] also attributed the lower SOC recovery percentage in WB method to more stable OC compounds in the mineral fraction. It is believed that part of the soil organic matter is stabilized by clay minerals through mineral organic binding[16] and SOC particle size determines its retention mechanism. Colloidal forms are retained by sorption, while particulate organic matter can occur outside or

Table 1. Summary of soil organic carbon (%) as obtained from WB method and TOC analyser

Attributes	SOC (%)		Clay (%)
	Walkley and Black method	TOC analyser	
Minimum	0.23	0.47	30.0
Maximum	2.00	2.80	78.0
Average	0.88	1.29	57.3
Standard deviation	0.37	0.48	10.2

inside aggregates[17]. Thus, the recovery of OC in WB method decreases with increase in clay content however, in TOC the effect is negligible due to complete oxidation of carbon materials at higher temperature (Figure 2). Nevertheless, WB method follows similar trend in OC recovery with increase in clay content. This has resulted in varying relationship between WB and TOC analyser which are plotted in Figure 3. The soils are grouped into classes based on clay content, viz. 30–50, 50–70 and more than 70% and gave R^2 value of 0.88, 0.79 and 0.52 respectively. In general, textural class and pedogenetic horizon showed significant differences in SOC recovery. This necessitates the use of different conversion factors to convert WB estimates to TOC values in proportionate to clay content.

Grouping of samples based on land use types indicated that SOC content varied with land use as the amount of organic residues returned to the soil is closely related to land use (Figure 4). The low recovery of SOC in WB method under different land use may be due to the nature of organic materials especially lignin content which reduces oxidation of organic materials. In case of agricultural soils, the SOC estimates ranged from 0.37 to 1.11% by WB method and from 0.79 to 1.60% by TOC analyser. Plotting these estimates indicated a high positive correlation ($R^2 = 0.86$). In forest soils, the SOC varies from 0.59 to 2.00% and 0.78 to 2.71% by WB and TOC respectively. A high correlation ($R^2 = 0.91$) is recorded between the two methods. It is also observed that the average clay content in forest soils is 55%, which is lower than the agricultural soils. Similar trend in OC recovery was observed in wasteland but it recorded the lowest correlation ($R^2 = 0.66$). The wasteland mostly contains organic matter and residues derived from bushes and shrubs of different kinds. It is also observed that with increase in OC (>1.5%), the recovery by WB method decreases which seriously undermines the reliability of forest carbon, especially estimates in *Mollisols* (soil order). De Vos

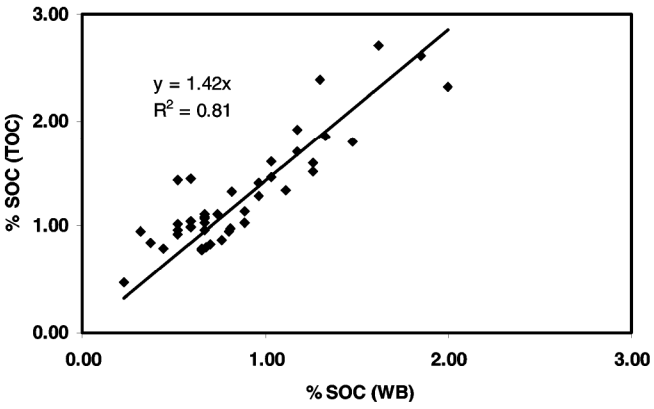

Figure 1. Comparison of Walkley and Black technique (WB) and total organic carbon (TOC) analyser in soil organic carbon (SOC) recovery.

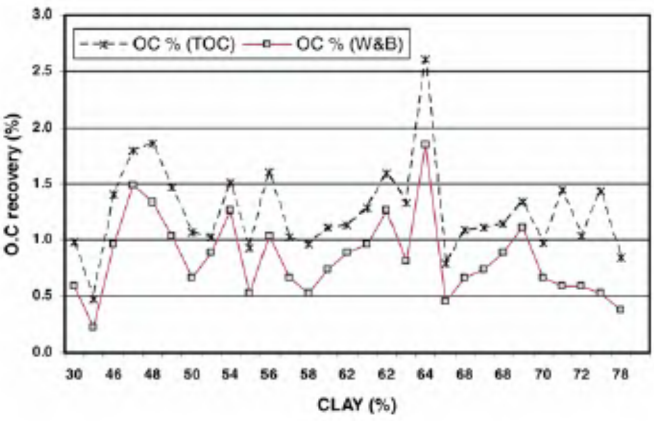

Figure 2. Soil organic carbon recovery as influenced by clay content in WB and TOC analyser.

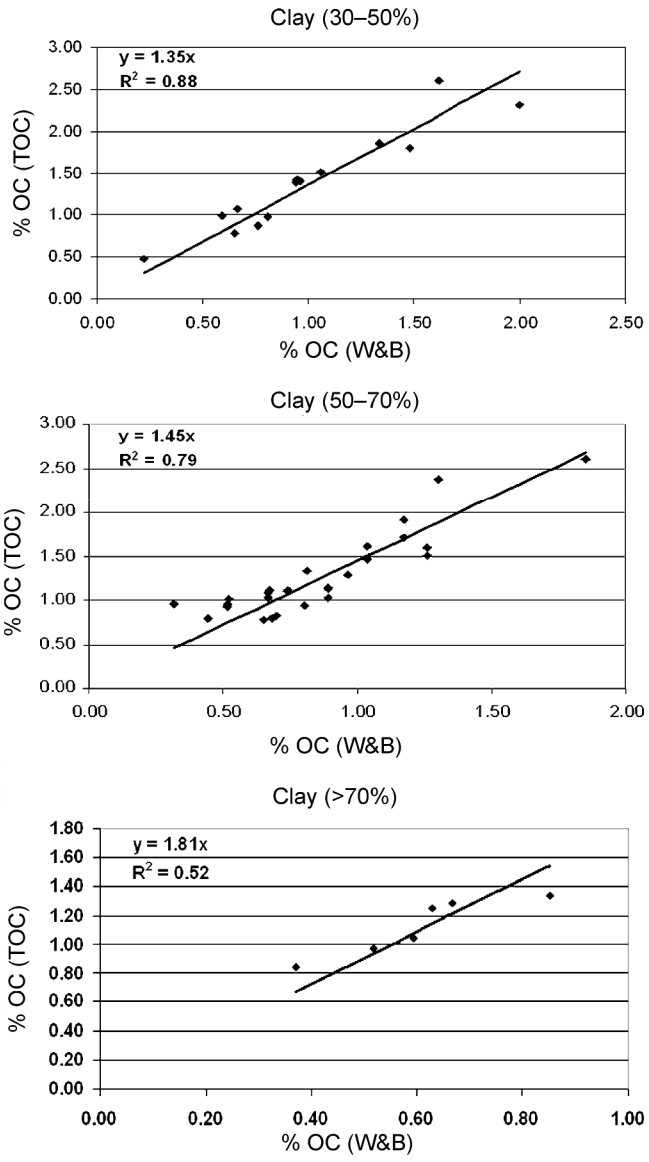

Figure 3. Soil organic carbon recovery (%) in WB and TOC with increasing clay content.

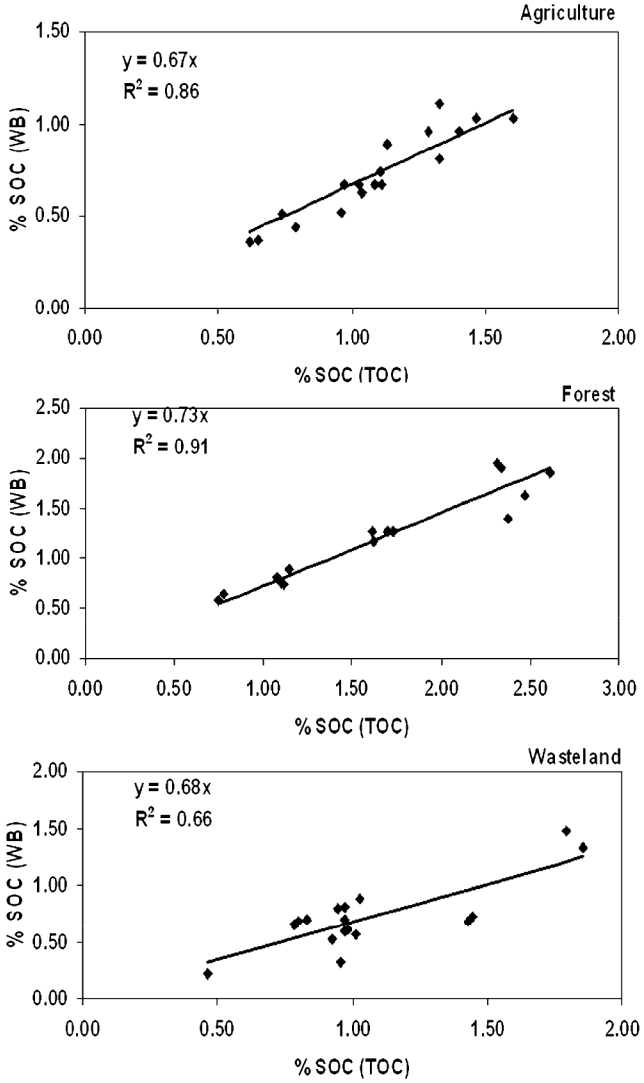

Figure 4. Soil organic carbon recovery (%) in WB and TOC under different land use.

et al.[6] partly attributed the low recovery from samples to charcoal and resistant carbon particles. Zhao *et al.*[18] also found significantly higher correlation between the proportion of clay and silt sized particles and the amount of SOC associated with this fraction in corn field than other soils.

The percentage of carbon recovered by the WB method was found to be systematically lower than commonly accepted values. However, a good linear relationship with TOC enabled acceptable prediction of SOC which was most precise when using the original WB method. Hence, we have made an attempt to determine conversion factors for soil TOC to allow the conversion of data generated through conventional soil survey programme and current estimates based on the wet digestion method to values equivalent to dry combustion (TOC analyser) which gives the highest C recovery. In general, it is recommended to use a correction factor of 1.42 to convert WB method estimates into TOC values. If clay content is known, correction factor of 1.35, 1.45 and 1.81 can be used for clay content percentage of 30–50, 50–70 and more than 70 respectively. Similar attempts have been made in Australia for nation carbon accounting system[19]. While using these conversion factors, land use and type of organic residues should be kept in mind. Hussain and Olson[15] also proposed a common correction factor of 1.41 for OC analysis with WB method in mineral soils. The correction factor was estimated at 1.58 instead of 1.30–1.35 by De Vos *et al.*[6] for forest SOC assessment.

In order to show the need for such a correction factor in SOC pool estimation, we have used the current data sets and estimated the SOC stock[20]. SOC stock Qi (Mg m^{-2}) in a soil layer or sampling level i with a depth of Ei (m) depends on the carbon content Ci (gC g^{-1}), bulk density Di (Mg m^{-3}) and on the volume fraction of coarse elements Gi, given by the formula:

$$Qi = Ci\,Di\,Ei(1 - Gi). \quad (1)$$

The results indicated that the study area has soil C stock of 260.76 and 312.9 terra gram up to 0–50 cm by WB method and TOC analyser respectively. This is equal to 20% under estimation of soil C stock by WB method. The results of the present study have implications in the reported SOC pool size in forest and agricultural soils of India and temporal changes. In order to use WB method, carbon data or historical data bases for national carbon inventory, laboratory and method-specific determinations of the recovery rate using a total analyser are strongly recommended.

1. Schlesinger, W. H., *Biochemistry: An Analysis of Global Change*, Academic Press, New York, 1991, p. 443.
2. Lal, R., Kimble, J. M., Follettt, R. F. and Cole, C. V., *The Potential of US Cropland to Sequester Carbon and Mitigate the Greenhouse Effect*, Ann Arbon Press, Chelsea, MI, p. 128.
3. Bhattacharyya, T., Pal, D. K., Mandal, C. and Velayutham, M., Organic carbon stock in Indian soils and their geographical distribution. *Curr. Sci.*, 2000, **79**, 655–660.
4. Chhabra, A., Palria, S. and Dadhwal, V. K., Soil organic carbon pool in Indian forests. *Forest Eco. Manage.*, 2003, **173**, 187–199.
5. Jha, M. N., Gupta, M. K., Saxena, A. and Kumar, R., Soil organic carbon store in different forest of India. *Indian Forester*, 2003, **129**, 714–724.
6. De Vos, B., Lettens, S., Muys, B. and Deckers, J. A., Walkley–Black analysis of forest soil organic carbon recovery, limitations and uncertainty. *Soil Use Manage.*, 2007, **23**, 221–229.
7. Walkley, A. and Black, I. A., An examination of Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.*, 1934, **34**, 29–38.
8. Rhodes, E. R., Kamara, P. Y. and Sutton, P. M., Walkley–Black digestion efficiency and relationship to loss on ignition for selected Sierra Leone soils. *Soil Sci. Soc. Am. J.*, 1981, **45**, 1132–1135.
9. Chacon, N., Dezzo, N., Folster, H. and Mogollon, P., Comparison between colorimetric and titration methods for organic carbon

determination in acidic soils. *Commun. Soil Sci. Plant Anal.*, 2002, **33**, 203–211.
10. Soon, Y. K. and Abboud, S., A comparison of some methods for soil organic-carbon determination. *Commun. Soil Sci. Plant Anal.*, 1991, **22**, 943–954.
11. Mikhailova, E. A., Noble, R. R. P. and Post, C. J., Comparison of soil organic carbon recovery by Walkley–Black and dry combustion methods in the Russian Chernozem. *Commun. Soil Sci. Plant Anal.*, 2003, **34**, 1853–1860.
12. Diaz, Z. M., Soil carbon recovery by Walkley–Black method in Typic Hapludoll. *Commun. Soil Sci. Plant Anal.*, 1999, **30**, 739–745.
13. Skjemstad, J. O. and Taylor, I. A., Does the Walkley–Black method determine soil charcoal? *Commun. Soil Sci. Plant Anal.*, 1999, **30**, 2299–2310.
14. Day, P. R., Particle fractionation and particle-size analysis. In *Methods of Soil Analysis* (ed. Black, C. A.), American Society of Agronomy, Madison, WI, 1965, pp. 545–567.
15. Hussain, I. and Olson, K. R., Recovery rate of organic C in organic matter fractions of Grantsburg soils. *Commun. Soil Sci. Plant Anal.*, 2000, **31**, 995–1001.
16. Koekkoeke, J. W., Van Genuchten, P. P. L., Buurman, P. and Van Lagen, B., Amount and composition of clay associated soil organic matter in a range of kaolinitic and smectitic soils. *Geoderma*, 2001, **99**, 27–49.
17. Zinn, Y. L., Lal, R., Bigham, J. M. and Resck, V. S. D., Edaphic controls on soil organic carbon retention in the Brazilian Cerrado: texture and mineralogy. *Soil Sci. Soc. Am. J.*, 2007, **71**, 1204–1214.
18. Zhao, L., Sun, Y., Zhang, X., Yang, X. and Drury, C. F., Soil organic carbon in clay and silt sized particles in Chinese mollisols: relationship to the predicted capacity. *Geoderma*, 2006, **132**, 315–323.
19. Skjemstad, J. O., Spouncer, L. R. and Beech, A., National carbon accounting system, Technical Report, 2000, p. 15.
20. Batjets, N. H., Total carbon and nitrogen in the soils of the world. *Eur. J. Soil Sci.*, 1996, **47**, 151–163.

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Introgression of pink bollworm resistance from wild *Gossypium thurberi* Tod. to cultivated *Gossypium arboreum* L. cotton: pre-breeding efforts

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Pink bollworm (PBW, *Pectinophora gossypiella* Saund.) is a pest of economic importance in cotton producing countries. Earlier results of host-plant resistance to the PBW indicated races of *Gossypium hirsutum* L. and some wild species including *Gossypium thurberi* Tod. which are resistant to PBW. Hence prebreeding efforts were made to transfer PBW resistance from *G. thurberi* to *G. arborum*. F_1, F_2 and BC_1 populations along with parents were studied for morphological, boll anatomical characters and infestation by PBW larvae. The F_1, F_2 and BC_1 generations were distinct from both the parents for all morphological characters. The highest boll toughness was found in *G. thurberi*, followed by F_1, whereas it was lowest in *G. arboreum*. Significant negative correlation was found between toughness with locule damage by PBW larvae. Promising prebreeding material is identified which will be excellent source of developing lines combined with PBW resistance, fibre strength and drought tolerance.

Keywords: *Gossypium hirsutum* L., *Gossypium thurberi* Tod., *Pectinophora gossypiella*, pink bollworm, wild species.

COTTON fibre is an important raw material for the textile industry. Of the several pests attacking cotton pink bollworm (PBW), *Pectinophora gossypiella* Saunders is a serious pest of substantial economic importance in cotton producing countries[1].

Several species of *Gossypium*[2,3] including its taxonomic races are resistant to this pest. The presence of unidentified antibiotic factors in certain strains of upland cotton[4] provides significant resistance to PBW[3].

PBW infestation in the bolls of *G. thurberi* (*Thurberia thespesioides*) was lower than 4% as against 100% in cultivated cotton grown nearby[5]. *G. thurberi* has been reported to be either free from the attack of PBW or its complete absence in Trinidad, Brazil and in the Anglo-Egyptian Sudan, although the neighbouring commercial cotton had 97% attack[6]. Immunity of *G. thurberi* and its hybrid *G. arboreum* × *G. thurberi* is due to some repellant scent present especially in the petals, that prevents the oviposition by the moth on the plant[7] in addition to other characters, viz. smallness of bolls, smoothness of boll surface, repellant and unpalatable principle in the seed[7,8]. Bolls of *G. thurberi* were essentially ignored by the PBW when grown near a commercial cultivar of *G. hirsutum*[5] probably because of the extremely small bolls of *G. thurberi*, that increased mortality and extremely slower growth rates that were due to lack of food. Further, the resistance in *G. thurberi* is due to tight-fitting calyx with triangular lobes and flared bracts causing either escape or non-preference. In addition to *G. thurberi*, *G. stocksii* showed similar resistance but had very small bolls[5].

Despite several attempts made by the cotton breeders to incorporate PBW resistance of *G. thurberi* in the new world cottons, limited success has been reported[9]. In an attempt to transfer bollworm immunity from *G. thurberi* to upland cottons[10], a synthetic tetraploid F_1 *thurboreum*,

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