Short Communication

A note on pathogenic variations among isolates of *Colletotrichum graminicola* causing anthracnose disease of sorghum

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Key words: Anthracnose, *C. graminicola*, pathotype, sorghum, virulence diversity

Anthracnose caused by *Colletotrichum graminicola* is one of the most devastating diseases of sorghum. The disease appears in severe form on forage sorghum under northern Indian conditions where yield losses up to 50% or more can occur (Thakur *et al*., 2007). Use of resistant cultivars as a strategy to control the disease has often met with limited success because of the breakdown of resistance. The pathogen is highly variable and known to have physiological races (Mathur *et al*., 2000). Pande *et al*. (1991) studied 9 isolates of *C. graminicola* from India and observed large genetic variation present in them. To develop a sustainable anthracnose resistance breeding programme, information on the present state of variation in the pathogen population is essential. There is no report of variability study in sorghum anthracnose for almost last two decades. The objective of the present investigation was to study the virulence diversity among different isolates of *C. graminicola* causing anthracnose of sorghum.

Ten cultures which included five isolates from Udham Singh nagar (UKL-Uttarakhand Mandawali isolate, UKK-Uttarakhand Kichha isolate, UKN- Uttarakhand Gadarpur isolate, UKA- Uttarakhand Pantnagar isolate, UKF-Uttarakhand Sitarganj isolate), two isolates from Haridwar (UKD-Uttarakhand Patanjali isolate, UKS-Uttarakhand Bahadarabad isolate), two isolates from DehraDun (UKB-Uttarakhand Doiwala isolate, UKC-Uttarakhand Dhakrani isolate) and one isolate from Nainital (UKH-Uttarakhand Haldwani isolate) were obtained from a repository of *C. graminicola* isolates available at sorghum pathology laboratory of the Department of Plant Pathology, GBPUA&T, Pantnagar. The cultures that showed morphological and cultural variations in the laboratory and pathogenic variations under glass house conditions were further evaluated under field conditions during two consecutive kharif seasons of 2013 and 2014 using a set of ten selected cultivars/lines. Sorghum lines grown in a plot of 2 rows of 4 m length spaced at 45x15 cm in RBD with three replications were spray inoculated with spore suspension of each isolate @1x10^6 conidia ml^{-1} at 15-and 30 days after sowing (DAS). Observations on disease severity were taken 15 days after inoculation and at 50% flowering using a continuous 1-9 scale where: 1= <1% leaf area covered; 2=1-5% leaf area covered; 3=6-10% leaf area covered; 4=11-20% leaf area covered; 5=21-30% leaf area covered; 6=31-40% leaf area covered; 7=41-50% leaf area covered; 8=51-75% leaf area covered; 9=76-100% leaf area covered (Thakur *et al*, 2007). Analysis of the data was done using GBPUAT Analytical Programme STPR-723 for pooled analysis.

The isolates produced typical disease symptoms on plants. On the basis of type of symptoms produced on foliage and the virulence, the isolates could be differentiated into seven categories (Table 1).

Isolates UKK, UKC and UKH were very similar in pathogenicity. At 15 DAS these isolates did not infect IRAT 204, IS 2312, CSV 21F and PC 5. These isolates produced black dot like acervuli on the foliage of Kekri local. These isolates were therefore, considered one pathotype. Isolates UKB and UKN were found to be similar in pathogenicity. These isolates were similar to isolates UKK, UKC and UKH in not infecting IS 2312 but unlike these isolates they failed to infect IS 18442 and IS 3089 at 15 DAS. Isolate UKL could not infect K local, IS 18442, IRAT 204, SSG 59-3 and PC 5 at 15 DAS but resulted in severe mid rib infection in K local and CSV 21F in addition to leaf spots at 30 DAS, thus considered as a different pathotype. Isolate UKD did not infect IS 8354 and CSV 21 F at 15 DAS. Isolate UKA like Isolate UKL could not infect K local initially but it was distinct from Isolate UKL in infecting other cultivars at 15 DAS. Isolate UKF also varied in virulence on different cultivars on inoculation at 15 DAS. Isolate UKS infected only K local, IS 3089, R local and SSG 59-3 at 15DAS and was considered a separate pathotype. However, every isolate infected all the cultivars used in the investigation to a varying extent when inoculated at 30 DAS.

In previous studies on pathogenic variability, anthracnose

Table 1: PDI and putative pathotyping of *Colletotrichum graminicola* isolates on sorghum lines

Isolates	PDI (Pooled mean of 2013 and 2014 crop season)																				Acervulus production/ midrib infection	Putative Patho-typing
	Sorghum lines																					
	K Local		IS8354		IS18442		IRAT204		IS2312		IS3089		CSV21F		R Local		SSG59-3		PC5			
	I*	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II		
UKL	0.00	80.33	30.34	74.83	0.00	63.33	0.00	52.17	29.33	70.17	27.34	73.33	22.84	67.33	33.00	80.83	0.00	64.00	0.00	40.17	Severe midrib infection in K Local and CSV21F	P1
UKK	29.00	79.33	28.33	67.33	25.50	65.50	0.00	66.00	0.00	60.17	27.50	74.17	0.00	65.17	32.00	73.00	26.67	69.67	0.00	40.00	Numerous acervuli on K Local	P2
UKD	33.17	73.50	0.00	69.67	30.84	71.17	27.17	66.17	28.34	63.50	33.50	78.33	0.00	63.17	33.83	73.67	32.50	71.17	22.84	41.17	Nil	P3
UKN	29.17	80.50	30.50	77.83	0.00	72.50	27.00	66.33	0.00	63.83	0.00	65.83	28.84	63.50	30.50	73.00	25.84	66.17	24.17	42.17	Nil/Slight	P4
UKS	33.67	77.83	0.00	73.00	0.00	70.67	0.00	61.67	0.00	65.50	32.17	78.00	0.00	60.17	34.84	73.50	33.83	62.00	0.00	39.83	Nil	P6
UKA	0.00	75.17	32.00	82.67	31.84	73.17	29.50	67.50	0.00	66.33	0.00	73.33	0.00	59.33	34.40	75.00	29.50	66.00	0.00	40.17	Nil	P5
UKF	33.50	75.00	0.00	78.87	0.00	67.17	30.33	64.67	32.50	65.00	0.00	81.00	26.34	57.50	34.84	80.83	0.00	59.67	24.50	37.83	Nil	P7
UKB	31.34	79.17	30.83	78.50	0.00	67.33	28.67	66.00	0.00	63.33	0.00	75.33	28.67	62.50	29.34	69.33	26.00	67.67	21.33	37.33	Nil/Slight	P4
UKC	27.84	77.83	30.00	79.50	30.50	67.00	0.00	61.50	0.00	66.50	35.17	73.83	0.00	65.50	36.50	71.17	27.83	62.33	0.00	37.17	Numerous acervuli on K Local	P2
UKH	34.17	74.33	27.00	78.17	28.83	67.33	0.00	64.33	0.00	63.50	30.67	73.67	0.00	67.00	33.00	70.17	23.84	59.83	0.00	36.83	Numerous acervuli on K Local	P2
CD at 5%	**1.75**	**1.65**	**1.75**	**1.74**	**1.35**	**1.60**	**1.49**	**2.89**	**1.03**	**1.47**	**1.38**	**1.44**	**1.17**	**1.29**	**1.92**	**2.27**	**1.97**	**1.59**	**1.71**	**1.36**	**-**	**-**
CV	**5.95**	**1.82**	**7.49**	**1.96**	**7.82**	**2.00**	**8.89**	**2.16**	**9.77**	**1.93**	**6.34**	**1.64**	**9.40**	**1.74**	**4.94**	**1.97**	**7.45**	**2.09**	**15.77**	**2.95**	**-**	**-**

*I = PDI when inoculated 15 DAS, II = PDI when inoculated 30 DAS

virulence was studied only at 6 leaf stage (Thakur *et al*., 1999). Mathur and Totla (2001) used three parameters for differentiating seven isolates of *C. graminicola* on six sorghum cultivars under glass house conditions and found additional parameters useful for finer differentiation. There are some reports suggesting that anthracnose symptoms do not develop on plants below 6-leaf stage i.e. 25 DAS (Nakamura, 1985; Ferreira and Casela, 1986; Thakur, 1995) probably due to high HCN contents in very young seedlings which may get diluted with plant growth. Similarly, in our studies plants at 30 DAS developed symptoms more readily but as the isolates could infect the seedlings of some cultivars at 15 DAS, it is likely that these isolates have the ability to overcome HCN in those lines. It has been demonstrated that *Gloeocercospora sorghi* causing zonate leaf spot of sorghum could overcome HCN by hydrolysing it with an enzyme Formamide hydrolyase (Myers and Fry, 1978). However, whether such a mechanism is used by these isolates of *C. graminicola* needs further investigation.

The study indicated prevalence of pathogenic variability among the isolates of *C. graminicola* which could prove valuable for designing the management strategies for the disease and to screen resistant germplasm for breeding programme.

REFERENCES

Ferreira, A.S. and Casela, C.R. (1986). Races pathogenicas de *Colletotrichum graminicola* agente causal de anthracnose em sorgho (*Sorghum bicolor* (L.) Moench). *Fitopatol Brasil*., 11: 833-87.

Mathur, K., Thakur, R.P. and Rao, V.P. (2000). Pathogenic variability and vegetative compatibility among isolates of *Colletotrichum graminicola* and *C.gloeosporiodes* causing foliage and grain anthracnose in sorghum. *Indian Phytopath.,* 53: 407-414.

Mathur, K. and Totla, K.G. (2001). Virulence diversity among isolates of *Colletotrichum graminicola* infecting foliage, stalk and grains of sorghum. *J. Mycol. Pl. Pathol*., 31 (1):67-71.

Myers, D.F. and W.E. Fry. (1978). Enymatic release and metabolism of hydrogen

cyanide in sorghum infected by *Gloeocercospora sorghi*. *Phytopathology*, 68: 1717-1722.

Nakamura, K. (1985). Especializacao fisiologica em *Colletotrichuim graminicola* (Ces.) Wils., agente causal de anthracnose do sorgho (*Sorghum* spp). Doctoral Thesis, Universidada Esta Paulista, Jabotical, Brazil, 143 p.

Pande, S., Mughogho, L.K., Bandhyopadhyay, R. and Karunakar, R.I. (1991). Variation in pathogenicity and cultural characteristics of sorghum isolates of *Colletotrichum graminicola* in India. *Plant Dis*., 75: 778-783.

Thakur, R.P., Reddy, B.V.S. and Mathur, K. (2007). Screening techniques for sorghum diseases. Information Bulletin no. 76. Patencheru 502324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 92 p.

Thakur, R.P., Mathur, K., Rao, V.P., Chandra, S., Shivramkrishnan, S., Kanna, S., Hiremath, R.V., Tailor, H.C., Kushwaha, U.S., Dwivedi, R.R. and Indira, S. (1999). Pathogenic and genetic characterization of six Indian populations of *Colletotrichum sublineolum*, the causal agent of sorghum anthracnose. *Indian Phytopath*., 51: 321-323.

Thakur, R.P. (1995). Status of international sorghum anthracnose and pearlmillet downy mildew virulence nurseries. In: Disease Analysis through Genetics and Biotechnology-Interdisciplinary Bridges to Improve Sorghum and Millet Crops (J.F. Leslie and R.A. Frederiksen, eds.) Iowa State University Press, Iowa, Ames., Pp 75-92.

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A note on host diversity of *Criconema* spp.

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Key words: *Criconema,* host diversity, host range, Nematode

Nematode species of the genus *Criconema* (Tylenchida: Criconemitidae) are widely distributed and parasitize many plant species from very primitive orders to advanced ones. They are migratory ectoparasites and feed on root tips or along more mature roots. Reports like Rathore and Ali (2014) and Rathore (2017) reveal that most nematode species prefer feeding on plants of certain taxonomic group (s). In the present study an attempt has been made to precisely trace the host plant affinity of twenty-five *Criconema* species feeding on diverse plant species. Host species of various *Criconema* species reported by Nemaplex (2018) and others in literature were aligned with families and orders following the modern system of classification, i.e., APG IV system (2016). According to this system, angiosperms are classified in different clades and clades into different orders and subsequently into different families. Affinity of each *Criconema* species with its host plants was numerically determined by calculating General Association Index (GAI), and for a group of species in a particular clade(s) by having Specific Association Index (SAI) following the system of Rathore and Tiwari (2016). The status of nematode species was further supported by the classification of Berneys and Chapman (1994).

Association and affinity of 25 *Criconema* species presented in Table 1 revealed that 35.35 % plants were preferred in Rosids followed by monocots (25.252 %) and Asterid (20.202 %). Though, Rosids and Asterids are different clades but both possess dicotyledonous plants. The combination of two clades proves that dicotyledons showed preference over monocots. Superrosids and Superasterids were represented by a few host plants only. However, Magnoliids and Gymnosperms substantially contributed in the host range of this nematode species. Though Rosids revealed greater preference over Asterids, the percent host families and orders were similar in number as reflected by similar SAI values. The SAI value was slightly higher for monocots that indicate stronger affinity. The same was higher for gymnosperms (0.467) in comparison to Magnolids (0.413) (Table 1).

Perusal of taxonomic position of host species in Table 2 revealed that 68 % of *Criconema* spp. were monophagous and strictly fed on one host species. Of these, 20 % from Magnoliids were monophagous (*C. acriculum, C. grassiator, C. karacsi, C. magnolia, C. petasum*); 20 % from Rosids (*C. demani, C. featherensis, C. mangifarae, C. parmistum C. ravidum*); 12 % from Asterids (*C. annulifer, C. acanum, C. celetum*); 12 % from monocots (*C. pauciannulatum, C. quasiclemani, C. warrenense*) and 4 % from gymnosperms (C. *neoaxestis*). Twenty-eight percent *Criconema* spp. were polyphagous and one oligophagous. GAI was 1.0 for all monophagous and oligophagous species, whereas the same was less than 1 for polyphagous species. Rosids contributed in the host range of all the polyphagous *Criconema* spp., while the association of host plants from other clades was more or less 50 %.

Maximum numbers of *Criconema* spp. were harboured by host families like Lauraceae (5), Magnoliaceae (5) in Magnolids; Poaceae (5) in monocots; Fagaceae (4) and Rosaceae (4) in Rosids and Pinaceae (3) in gymnosperms.

Table 1: **Association of *Criconema* species to different host parameters**

Taxonomic clades	Host species	Host genera	Host families	Host orders	SAI
Magnoliids	10 (10.101)	10 (10.753)	10 (14.705)	9 (15.789)	0.413
Monocots	25 (25.252)	25 (26.882)	14 (20.588)	11 (19.298)	0.54
Superrosids	1 (1.010)	1 (1.075)	1 (1.470)	1 (1.754)	1
Rosids	35 (35.353)	30 (32.258)	22 (32.353)	19 (33.334)	0.52
Superasterids	3 (3.050)	3 (3.226)	3 (4.412)	1 (1.754)	0.714
Asterids	20 (20.202)	19 (20.430)	13 (19.118)	11 (19.298)	0.512
Gymnosperms	5 (5.000)	5 (5.376)	5 (7.353)	5 (8.772)	0.467

Figures in parentheses are per cent values; SAI=Specific Association Index

Table 2: Taxonomic position of host plants of*Criconema* spp.

S. No.	*Criconema*spp.	Host species	No. of hostspecies	GAI	Status
1	*C. acriculum*	Magnoliids: Lauraceae (1)*Umbellularia californica*	1	1	Monophagous
2	*C. annulifer*	Asterids: Aquifoliaceae (1) *Ilex aquifolium*	1	1	Monophagous
3	*C. acanum*	Asterids: Asteraceae (1) *Solidago* sp.	1	1	Monophagous
4	*C. arkaense*	Monocots-Poaceae (2) *Arrhenatherum sp., Paspalum* sp.:Rosids: Cannabaceae (1) *Celtis accidentalis,*Sapindaceae (1) *Acer saccharum*	4	0.6	Polyphagous
5	*C. celetum*	Asterids: Gesneriaceae (1)*Saintpaulia* sp.	1	1	Monophagous
6	*C. crotaloides*	Magnolids: Lauraceae (1) *Umbelluria californica;* Rosids:Rosaceae (1) *Rubusparviflorus*; Asterids: Ericaceae (1) *Arctostaphylos manzanit*a; Gymnosperms:Pinaceae (1) *Pseudotsuga menziesii*	4	0.5	Polyphagous
7	*C. demani*	Rosids: Betulaceae (1) *Betula papyrifera*	1	1	Monophagous
8	*C. featherensis*	Rosids: Vitaceae (1) *Vitis californica*	1	1	Monophagous
9	*C. giardi*	Magnoliids: Lauraceae (1) *Persea americana*, Magnoliaceae (1) *Magnolia grandiflora*;Rosids: Moraceae (1) *Ficus carica*, Rosaceae (1)*Fragaria x ananassa*	4	0.6	Polyphagous
10	*C. grassator*	Magnoliids: Magnoliaceae *(1) Liriodendron tulipifera*	1	1	Monophagous
11	*C. kavacsi*	Magnoliids: Lauraceae (1) *Umbellularia californica*	1	1	Monophagous
12	*C. magnoliae*	Magnoliids: Magnoliaceae (1) *Magnolia grandiflora*	1	1	Monophagous
13	*C. mangiferum*	Rosids: Anacardiaceae (1) *Mangifera indica*	1	1	Monophagous
14	*C. mutabile*	Magnoliids:Lauraceae (1) *Persea americana*, Monocots: Araceae (1) *Philodendron* sp., Arecaceae (1) *Palmaceae* sp., Asparagaceae (1) *Yucca* sp., Bromeliaceae(2) *Billbergia* sp., *Bromeliaceae* sp.. Dioscoreaceae (1)*Dioscorea* sp., Musaceae (1) *Musa* sp., Poaceae (10)*Arrhenatherum* sp., *Avena sativa, Axonopu*s sp*., Bambusa* sp., *Cynodon dactylon, Echinchloa sp., Hordeum vulgare, Sorghum bicolor, Zea mays, Zoysia p.,*Typhaceae (1) *Typha* sp., Zingiberaceae (1) *Zingiber* sp.,Superasterids: Amaranthaceae (1) *Beta vulgaris,*Cactaceae (1)*Cactaceae* sp., Nyctaginaceae (1) *bougainvillea* sp.; Rosids: Fabaceae (3) *Medicago sativa, Trifoleum repens, Vigna unguiculata,* Juglandaceae (2)*Juglanshendsii, Juglans* sp., Malvaceae (1) *Gossypium hirsutum*, Moraceae (1)*Morus* sp., Rosaceae (8)*Fragariachiloensis, Malus sylvestris, Prunus domestica, Prunus dulcis, Prunus persica, Pyracantha sp., Pyrus communis, Rosa* sp., Rutaceae (2) *Citrus sinensis, Citrus* sp., Sapindaceae (1*) Acer* sp.; Vitaceae (1) *Vitis vinifera* Superrosids: Altingiaceae (1) *Liquidamber* sp.;Asterids: Acanthaceae (1) *Acanthus* sp., Aquifoliaceae(1) *Ilex* sp., Araliaceae (1) *Aralis* sp., Asteraceae (5)*Arctiumlappa, Baccharis sp., Dahlia sp. Tagetes erecta, Tagetes* sp., Convolvulaceae (2) *Dicondra* sp., *Ipomoea batatas*, Ericaceae (1) *Rhododendron* sp., Oleaceae (2) *Ligustrum* sp., *Syringea* sp., Solanaceae (2*) Nicotiana* sp*., Solanum lycopersicum*, Theaceae (1) *Camellia* sp.Gymnosperms: Pinaceae (1)*Pinus*sp	60	0.564	Polyphagous
15	*C. neoaxestis*	Gymnosperms: Pinaceae (1) *Cedus lebani*			
16	*C. pauciannulatum*	Monocot: Poaceae (1)*Zea mays*	1	1	Monophagous
17	*C. permistum*	Rosids: Vitaceae (1) *Vitis vinifera*	1	1	Monophagous
18	*C. petasum*	Magnoliids: Magnoliaceae (1) *Liriodendron tulipifera*	1	1	Monophagous
19	*C. quasiclemane*	Monocot: Cyperaceae (1) *Scirpus americanus*	1	1	Monophagous
20	*C. ravidum*	Rosids: Fagaceae (1)*Quercus* sp.	1	1	Monophagous
21	*C. sphagni*	Magnoliids: Magnoliaceae (1) *Liriodendron*	1	1	Monophagous

		tulipifera;Monocots: Poaceae (1) *Arrhenatherum* sp.;Rosids :Fagaceae (1)*Quercus* sp.	3	0.556	Polyphagous
22	*C. tribule*	Rosids: Fagaceae (2*) Fagus* sp.,*Quercus* sp.	2	1	Oligophagus
23	*C. vishwanathum*	Rosids: Rosaceae (2) *Prunus domestica, Prunus persica* Gymnosperms: Cupressaceae (1) Juniperus oxycedrus	3	0.833	Polyphagous
24	*C. warrenense*	Monocots: Poaceae (1)*Paspalum* sp.	1	1	Monophagous
25	*C. zantene*	Rosids: Fagaceae (1)*Quercus* sp.; Gymnosperms: Podocarpaceae (1)*Podocarpus* sp.	2	0.667	Polyphagous

C. mutabile parasitized maximum number of host species (Table 3).

Though *Criconema* spp. parasitize many varieties of host species, nevertheless they tend to prefer woody plants. To examine this issue further, all the host families except gymnosperms were aligned according to the classification of Hutchinson (1973). He classified angiosperms into monocotyledons and dicotyledons. Hutchinson divided monocotyledons into calyciferae (calyx bearers-with distinct (usually green) calyx and corolla), corolliferae (calyx and corolla are more or less similar), and glumiflorae (perianth is much more reduced or represented by lodicules), whereas dicotyledons were partitioned into Lignosae (fundamentally woody plants) and Herbaceae (fundamentally herbaceous group of plants). *Criconema* spp. parasitized plants from 28 families (Magnoliids, Superrosids, Rosids, Superasterids, Asterids) and according to Hutchinson's classification 21 aligned with Lignosae and 7 with Herbaceae indicating greater preference towards woody plants (75 %). It will be worthwhile to mention that among monocotyledons 50 % families had plants from Corolliferae. Family Poaceae was most dominating family in monocotyledons. Since, *Criconema* spp. showed greater preference towards woody plants, it is suggested that cultivated crops prone to these nematode species should be grown away from forest areas.

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REFERENCES

APG IV. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. APG IV: *Botanical Journal of the Linnean Society,* 181 (1): 1-20. (doi: 10. 1111/boj.12385)

Berneys, E.A. and Chapman, R.F. (1994). Host plant selection by phytophagous insects (Contemporary topic in entomology), New York, Springer.

Hutchinson, J. (1973). Families of flowering plants. 3rd ed. Oxford with Clarendon Press, 968p.

Rathore, Y.S. and Ali, S.S. (2014). Relationships of root-knot nematode, *Meloidogyne incognita*, with taxonomic groupings of host plants. *Trends in Biosciences,* 7 (13): 1562-1568.

Rathore, Y.S. (2017). Host preference in *Pratylenchus* species. *Trends in Biosciences,* 10 (32): 6747-6758.

Nemaplex (2018). Nemabase host range of genus and species of plant feeding.

Rathore, Y.S. and Tiwari, S.N. (2016). Relationships of different species of root-knot nematodes to their shost taxonomic groups. *International Journal of Plant, Animal and Environment Science,* 7 (1): 29-36.

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Form and preparation of manuscripts

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Authors are requested to submit manuscripts electronically. We strongly suggest that authors carefully check the final version of their manuscripts for compliance with the format and style of the journal. In order to simplify journal production and minimize the number of errors, the final draft of the paper should be submitted as an electronic file created by a word processor. Likewise, authors are urged to send figures, photographs, graphs, and tables electronically, using programs that generate interchangeable formats (Corel Draw, Excel).

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Manuscript should not exceed 30 typewritten pages, including figures and tables. Short communications and technical notes are limited to six type written pages including illustrations.

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Title, name(s) of the author(s), complete postal address/affiliation and e-mail address of the corresponding author only.

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The journal follows double blind review policy, therefore, the names or affiliations of authors should not be mentioned in the manuscript any where except on a separate 'Title Page'. The manuscript should consist of only Title of the article, Abstract, Keywords (3 to 6), Introduction, Materials and Methods, Results and/or Discussion, Conclusions, Acknowledgements and References.

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All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose potential interests on a separate 'Declaration of interest' page. If there are no interests to declare then please state this: 'Declarations of interest: none'. This summary statement will be ultimately published if the article is accepted.

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Manuscripts should be divided into sections and subsections by headings and subheadings up to a maximum of three levels. To

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Only SI quantities and units should be used (SI = Le Système International d'Unités). If data with non-SI units are reported, they should be put in parentheses behind the corresponding data with SI units. Symbols and abbreviations used to represent variables, constants, quantities, properties, etc. must be defined in the text at their first occurrence.

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Structural diagrams of molecules as well as mathematical equations should be drawn or written at the appropriate places in the manuscript in an extra line. Equations should be denoted by Arabic numerals (in parenthesis) toward the right-hand margin.

3.9 Acknowledgements

Acknowledgements of financial support, advice, and other kinds of assistance should be made at the end of the paper under the heading "Acknowledgements".

3.10 References

Citations in the main text should be given by the surname and year of publication. For example, McDonald (1962), Vera and Luzio (1987), Ellies *et al.* (1995; 1999) or: (McDonald, 1962; Vera and Luzio, 1987; Ellies *et al.*, 1995; 1999).

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3.10.1 Journal articles

Van Ranst, E., Utami, S. R., Vanderdeelen, J., Shamshuddin, J. 2004. Surface reactivity of Andisols on volcanic ash along the Sunda arc crossing Java Island, Indonesia. *Geoderma*, 123, 193-203.

3.10.2 Unpublished work

Papers that are unpublished but have been accepted must be cited with the journal's name followed by (in press). In all other cases, reference must be made to (unpublished work) or (personal communication).

3.10.3 Books and monographs

Tertian, R., Claisse, F. 1982. Principles of Quantitative X-Ray Fluorescence Analysis. Heyden, London, 385 p.

3.10.4 Chapters from multi-author books

Wold, S., Sjöström, M. 1977. Chemometrics, Theory and Application. In: B. R. Kowalski (ed). ACS Symposium Series N° 52. American Chemical Society, Washington, DC, pp: 243–282.

3.10.5 Theses

Hassink, J. 1995. Organic matter dynamics and N mineralization in grassland soils. Doctoral thesis, Wageningen University, The Netherlands, 250 p.

3.10.6 Patents

Miller, B.O. 1952. U.S. Patent 2542356, Dow Chemical Company; Chemical Abstracts 51 (1961) 2870.

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