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Genetic diversity, essential oil composition, and *in vitro* antioxidant and antimicrobial activity of *Curcuma longa* L. germplasm collections

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ABSTRACT

Genetic divergence was estimated among sixty-five genotypes of turmeric (*Curcuma longa* L.) using Mahalanobis D^2-statistics on thirteen agro-morphological quantitative traits. The genotypes were grouped into nine clusters. Cluster I had maximum number of genotypes (19) followed by cluster II (13), III (11), IV (5), V (4), VI (4), VII (4), VIII (3) and IX (2) in order. The genotypes which belongs to cluster VII had the maximum divergence (250.62), followed by cluster IX (244.61) and cluster VI (240.40). The highest inter-cluster distances were observed between cluster IX (1871.46) and cluster VIII (1296.51), suggesting that the genotypes included in these clusters may be used for future breeding programme. Traits like fresh weight of rhizome, dry weight of rhizome were the major contributors to the genetic divergence. The chemical composition of essential oils from eight selected genotypes of *Curcuma longa* L. was studied and identified by gas chromatography–mass spectrometry (GC/MS). The compounds cis-sesquisabinene hydrate (3.4%), curzerenone (0.6%), β-bisabolol (2.2%), and farnesol (1.2%) were found only in CIM Pitamber variety. The total percentage of compounds identified from the essential oil of *Curcuma longa* leaves was maximum in CIM Pitamber (98.1%) followed by Bhagauna (90.9%), Borai Khuwa (90%), TC 11 (86.9%), Bhagauna Baag (85.1%), CH 20 (84.3%), Paroraha JD Kola (75.4%) and JD Kola (74.8%). The antioxidant activity results demonstrated that *Curcuma* genotypes had marked ferric ions reducing ability and having electron donor properties for neutralising free radicals. All *Curcuma* genotypes exhibited bacteriostatic nature against *Mycobacterium smegmatis* strain, used for the screening of antitubercular activity.

1. Introduction

The *Curcuma longa* L. (Zingiberaceae), rhizome is commonly known as turmeric and used worldwide as a preservative, flavour and food-colouring agent. In turmeric rhizome two major classes of secondary metabolites i.e. phenolic curcuminoids and essential oil are present (Funk et al., 2010). The composition of the both metabolites depends on the nature of genotypes, environment, harvesting season, dry processing and storage conditions (Li et al., 2011). Because of its specific colour and flavour, the introduction of turmeric keeps the nutritional value and freshness of food items. Curcumin, a bright orange-yellow coloured crystalline compound is used as food colorant. As a food additive, turmeric improves the deliciousness, and shelf life of food products (Joe et al., 2004). According to the European Colour Directive, in food industry and regulatory authorities there is requirement of reliable validated techniques to determine the curcuminoids content for various ranges of food products (Scotter, 2009). The addition of turmeric in food product is required to obtain the desired colour intensity and sufficient glycine is added to reduce the bitterness (Goldscher, 1979). Sometimes dried pulverized turmeric is mixed with cereal husk (or hull) and sugars, inoculated with lactic acid bacteria and heat dried to reduce the bitterness of turmeric (Inafuku, 1996). For quantitative analysis of curcuminoids content numerous analytical methods have been reported by researchers. Some of the methods are based on spectrophotometric techniques, expressed as the total colour content of the sample.

Curcumin is an easily available non-toxic, natural substance inhibits pro-inflammatory markers (Anand et al., 2007). It is a keto-enol tautomeric compound having good properties as chelator of metal ions. It has predominant keto-form in acid or neutral solutions and the enol-form is predominant in alkaline solutions (Manolova et al., 2014). The major and the most studied curcuminoid found in turmeric is curcumin, which is recognized as the most responsible compound for the majority

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of beneficial effects. Besides curcumin, there are two more curcuminoids: demetoxycurcumin and bisdemetoxycurcumin. Turmeric powder, extracts and oleoresins are some of the widely used commercial products of *C. longa* plant. India is the largest producers, consumer and exporter of turmeric and its oleoresin (Sasikumar, 2005). Indians consume approximately 80–200 mg turmeric extract per day and total consumption is near about 480,000 ton annually (Deb et al., 2013). For pharmaceutical application, curcumin is an established ingredient which is expected to act as a major driver for market growth. In addition, due to increase in use of turmeric extract or curcumin in cosmetic formulation, it was expected to further augment market demand over the forecast period. The major secondary metabolites of turmeric such as oils and the diarylheptanoid curcumin are responsible for the pharmacological activities of turmeric powder, extracts and oleoresins. The main activities have been found to be antimicrobial, anti-inflammatory, anti-bronchial asthama hepatoprotective, wound healing, anticancer, anti-tumor, and anti-viral (Srimal, 1997). Generally, Gas chromatography–mass spectroscopy is a powerful tool for analysis of volatile oil, resolution of analytes was exclusively used as marker for optimization of the conditions. Curcumin possess excellent antioxidant properties (Wojdyło et al., 2007). It was reported to be more potent in preventing lipid peroxidation than alpha-tocopherol, pine bark extract, grape seed extract or the commonly used synthetic antioxidant BHT (Sreejayan and Rao, 1994; Majeed, 1995). A complex of the three curcuminoids was found to be more effective as an antioxidant than each of the components-curcumin, demethoxycurcumin, or bisdemethoxycurcumin used alone. The antioxidant activity of turmeric justified its use in a broad range of applications, including cosmetics (Thornfeldt, 2005), nutraceuticals and phytomedicines (Aggarwal and Harikumar, 2009). These medicinal attributes can be related to turmeric's high content of curcuminoids, especially curcumin, which is considered as a chemical marker of this species (Gupta et al., 2013). Essential oils are commercially important plant volatiles employed extensively in pharmaceutical, flavouring and perfumery industries and possess a wide range of pharmacological properties (Priya et al., 2012). The essential oil of *C. longa* has been well studied and reported to contain ar-turmerone, turmerone, turmerol and zingiberene as the major constituents. Essential oils from *C. longa* possess antioxidant, antimicrobial, anti-inflammatory and cytotoxic properties (Singh et al., 2002; Mau et al., 2003; Saccheti et al., 2005). Turmeric is sometimes added to oils as a preservative. The leaves of *C. longa* are aromatic in nature and contain essential oil. *Curcuma longa* leaves oil bestowed with medicinal values has been used for treatment of various ailments and many of its therapeutic properties have been experimentally validated including its antimicrobial activity (Tripathi et al., 2002). Both the curcumin and the oil have been shown to possess wound healing properties and inhibitory activities against pathogenic fungi both *in vitro* and *in vivo* (Srimal, 1997). Studies on genetic diversity of *Curcuma longa* and biological activity of essential oil would be beneficial in medicinal applications. The genotype characterization is a connecting link between conservation and plant genetic resource utilization. Genetic diversity is a raw material to the breeder because spectrum of available genetic variation determines the potential for selection and is useful in resolving phylogenetic relationships (Gupta et al., 2015). The genetic variation available in the population is responsible for the success of any plant breeding programme. It provides better chance to breeder for the selection of desired material (Gupta et al., 2011; Srivastava et al., 2017). In plants the essential oil production involves different biological processes carried by several genes which influenced by various factors such as heredity, growth and environment (Drew and Demain, 1977). In the present experiment genetic diversity has been find out among sixty five genotypes of *Curcuma longa* and biological activity of essential oils from eight plants has been studied. Essential oils was evaluated for phenolic content and radical scavenging activity using five different methods viz. DPPH radical scavenging assay, FRAP assay, total phenolic content determination, nitric oxide scavenging assay and total antioxidant

Table 1
Places of collection of sixty five genotypes of *Curcuma longa* L.

S. No.	Accessions	Rhizome colour	Places of collection
1	Roma Pantnagar	Orange	CRC-CIMAP, Pantnagar, Uttarakhand (India)
2	Roma	Orange	Calicut, (India)
3	83	Orange	CSIR-CIMAP, Lucknow U.P. (India)
4	Indo Persian	Orange	CSIR-CIMAP, Lucknow U.P. (India)
5	Bansal clone	Orange	CSIR-CIMAP, Lucknow U.P. (India)
6	CA20	Orange	CSIR-CIMAP, Lucknow U.P. (India)
7	JL55	Orange	CSIR-CIMAP, Lucknow U.P. (India)
8	CH31	Orange	CSIR-CIMAP, Lucknow U.P. (India)
9	CA67	Orange	CSIR-CIMAP, Lucknow U.P. (India)
10	CA6	Orange	CSIR-CIMAP, Lucknow U.P. (India)
11	CL72	Orange	CSIR-CIMAP, Lucknow U.P. (India)
12	CLL324	Orange	CSIR-CIMAP, Lucknow U.P. (India)
13	81	Orange	CSIR-CIMAP, Lucknow U.P. (India)
14	CA2	Orange	CSIR-CIMAP, Lucknow U.P. (India)
15	CH7	Orange	CSIR-CIMAP, Lucknow U.P. (India)
16	CH65	Orange	CSIR-CIMAP, Lucknow U.P. (India)
17	CH17	Orange	CSIR-CIMAP, Lucknow U.P. (India)
18	CH15	Orange	CSIR-CIMAP, Lucknow U.P. (India)
19	CH3	Orange	CSIR-CIMAP, Lucknow U.P. (India)
20	TC3	Orange	CSIR-CIMAP, Lucknow U.P. (India)
21	TC4	Orange	CSIR-CIMAP, Lucknow U.P. (India)
22	TC5	Orange	CSIR-CIMAP, Lucknow U.P. (India)
23	TC6	Orange	CSIR-CIMAP, Lucknow U.P. (India)
24	TC7	Orange	CSIR-CIMAP, Lucknow U.P. (India)
25	TC8	Orange	CSIR-CIMAP, Lucknow U.P. (India)
26	TC9	Orange	CSIR-CIMAP, Lucknow U.P. (India)
27	TC10	Orange	CSIR-CIMAP, Lucknow U.P. (India)
28	TC11	Orange	CSIR-CIMAP, Lucknow U.P. (India)
29	TC12	Orange	CSIR-CIMAP, Lucknow U.P. (India)
30	TC13	Orange	CSIR-CIMAP, Lucknow U.P. (India)
31	TC14	Orange	CSIR-CIMAP, Lucknow U.P. (India)
32	TC16	Orange	CSIR-CIMAP, Lucknow U.P. (India)
33	CH4	Orange	CSIR-CIMAP, Lucknow U.P. (India)
34	CH31	Orange	CSIR-CIMAP, Lucknow U.P. (India)
35	CH22	Orange	CSIR-CIMAP, Lucknow U.P. (India)
36	CH52	Orange	CSIR-CIMAP, Lucknow U.P. (India)
37	CH63	Orange	CSIR-CIMAP, Lucknow U.P. (India)
38	HOM60	Orange	CSIR-CIMAP, Lucknow U.P. (India)
39	NO 60	Orange	CSIR-CIMAP, Lucknow U.P. (India)
40	CH20	Orange	CSIR-CIMAP, Lucknow U.P. (India)
41	CH50	Orange	CSIR-CIMAP, Lucknow U.P. (India)
42	CH3	Orange	CSIR-CIMAP, Lucknow U.P. (India)
43	CH42	Orange	CSIR-CIMAP, Lucknow U.P. (India)
44	Pa1	Orange	Maharashtra, Mumbai, Maharashtra (India)
45	Boriwal	Yellow	Maharashtra, Mumbai, Maharashtra (India)
46	Panvel	Yellow	Maharashtra, Mumbai, Maharashtra (India)
47	Bha1	Orange	Maharashtra, Mumbai, Maharashtra (India)
48	Ma1	Red	Maharashtra, Mumbai, Maharashtra (India)
49	Bhagauna	Yellow	Bihar, West- Champaran, Maharashtra (India)
50	NKE1	Orange	Bihar, West- Champaran, Bihar (India)
51	NKE2	Orange	Bihar, West- Champaran, Bihar (India)
52	Lauria	Orange	Bihar, West- Champaran, Bihar (India)
53	Bhath	Orange	Bihar, West- Champaran, Bihar (India)
54	Bhagauna (Baag)	Orange	Bihar, West- Champaran, Bihar (India)
55	Paroraha JD Kola	Orange	Bihar, West- Champaran, Bihar (India)
56	JD Kola	Orange	Bihar, West- Champaran, Bihar (India)
57	Boraikhuwa	Orange	Assam, (India)
58	NDH13	Orange	N.D. University, Faizabad U.P. (India)

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