

Biochemical characterization of turmeric (*Curcuma longa* L.) germplasms and standardization of protocol for micropropagation of selected cultivars

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DEGREE OF DOCTOR OF PHILOSOPHY AGRICULTURE**

(Biochemistry)

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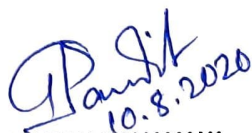
Dedicated
to
My Chairman,
Beloved parents
and Wife

Uttar Banga Krishi Viswavidyalaya
Department of Biochemistry
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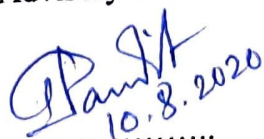

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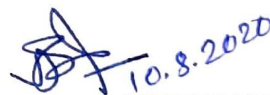
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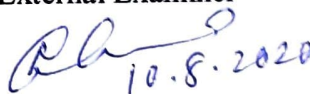
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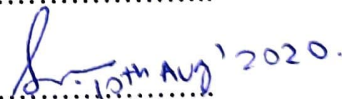

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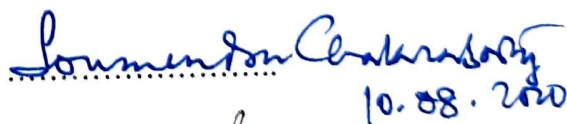
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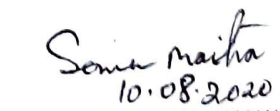

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CERTIFICATE

This is to certify that the work recorded in the thesis entitled “Biochemical characterization of turmeric (*Curcuma longa* L.) germplasms and standardization of protocol for micropropagation of selected cultivars” submitted by Mr. Sayan Chowdhury in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry of Uttar Banga Krishi Viswavidyalaya, is a faithful and bonafide research work carried out under my personal supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received during the course of investigation has been duly acknowledged.


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Sayan Chowdhury
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List of Abbreviations

Abbreviations	Full form
2,4-D	2,4-Dichlorophenoxyacetic acid
a*	Redness of colors
AAE	Ascorbic acid equivalent
ABTS	2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid
AD	Alzheimer's disease
AICRP	All India Coordinated Research project
ANOVA	Analysis of variance
AR	Analytical reagent
b*	Yellowness of colors
BA	Benzyl adenine
BAP	6-Benzylaminopurine
BHT	Butylated hydroxy toluene
BSA	Bovine serum albumin
CE	Catechin equivalent
Cu(I)-Nc	Copper neocuproine
CUPRAC	Cupric ion reducing antioxidant capacity
CuSO ₄	Copper sulphate
DAP	Days after planting
DMRT	Duncan's multiple range tests
DNA	Deoxyribonucleic Acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
ECV	Environmental coefficient of variation
EDTA	Ethylenediaminetetraacetic acid
FeSO ₄	Ferrous sulphate
FRAP	Ferric reducing antioxidant power
FYM	Farm yard manure
GAE	Gallic acid equivalent
GAM	Genetic advance mean
GCV	Genotypic coefficient of variation
h°	Hue angle
h ²	Heritability
H ₂ O ₂	Hydrogen peroxide
H ₂ R	H ₂ histamine receptors
HClO ₄	Perchloric acid
HIV	Human immunodeficiency virus
HNO ₃	Nitric acid
HPLC	High performance liquid Chromatography
IAA	Indole-3-acetic acid

Abbreviations	Full form
IBA	Indole-3-butyric Acid
IC ₅₀	Half maximal inhibitory concentration
K ₂ O	Potassium oxide
L*	Lightness of colors
LDL	Low density lipoprotein
MOP	Muriate of potash
MS	Murashige and Skoog
Na ₂ CO ₃	Sodium carbonate
NAA	α -Naphthaleneacetic acid
NaOH	Sodium hydroxide
NBT	Nitroblue tetrazolium
NMR	Nuclear Magnetic Resonance
NO ₂	Nitrogen dioxide
NPK	Nitrogen, Phosphorous, Potassium
OD	Optical density
OPC	Oligonucleotide purification cartridge
pM	Picomolar
P ₂ O ₅	Phosphorus pentoxide
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCV	Phenotypic coefficient of variation
PIC	Polymorphism information content
QE	Quercetin equivalent
RAPD	Random amplification of polymorphic DNA
RBD	Randomized block design
SOD	Superoxide dismutase
SRL	Sisco research laboratories
SSP	Single superphosphate
SSR	Simple sequence repeats
TAC	Total antioxidant capacity
TCP	Turmeric collection Pundibari
TDZ	Thidiazuron
TE	Trolox equivalent
UV	Ultraviolet
UV-VIS	Ultraviolet–visible spectrophotometer

Biochemical characterization of turmeric (*Curcuma longa* L.) germplasms and standardization of protocol for micropropagation of selected cultivars

Abstract

India is known as a country of spices where nearly sixty three spice crops are grown at various parts of the country. Turmeric (*Curcuma longa* L.), one of the most important and widely used spice belonging to the family *Zingiberaceae*, is a native of South-East Asia and grown widely in many parts of the tropical and subtropical regions. Curcumin and two of its closely related compounds namely demethoxycurcumin and bisdemethoxycurcumin are the principal coloring constituents of turmeric and are responsible for its wide array of biological and medicinal properties. A large number of turmeric genotypes grown in this region have not been scored properly based on their phenotypic and biochemical characters and also not been studied thoroughly for their detailed biochemical and nutritional composition as well as their enzymatic and antioxidant activities. The present study was focused on the scoring, estimation of biochemical attributes and micropropagation of the turmeric genotypes grown in terai region of West Bengal.

Curcumin content of sixty locally grown genotypes were measured and based on curcumin content, genotypes were divided into three groups having low to high curcumin namely Group I (0.10 - 1.00%), Group II (1.01 – 2.50%) and Group III (2.51- 5.50%). Best fifteen from each group were taken for further study. Phenotypic scoring of the forty five turmeric genotypes was done for three years (2015-16 to 2017-18) for plant height, number of tillers per plant, number of leaves per plant, leaf length, leaf breadth, clump weight and yield. All the forty five genotypes were analyzed for biochemical characters such as total protein, total phenol, essential oil content, iron content, rhizome colour and pH of the rhizome. The activity of the enzymes catalase, peroxidase and super oxide dismutase were measured along with total antioxidant activity.

From the present study it was found that genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2) and genetic advance mean (GAM) percentage were highest for average curcumin content. Very little difference between GCV and PCV was found for curcumin content followed by

iron content, leaf length, leaf breadth and plant height. PCV values were higher in all the cases than GCV values. Plant height, in particular, showed highly significant positive correlation with rhizome yield. Similar correlation was observed between curcumin and iron content also. Plant height and iron content exhibited significantly positive direct effect on curcumin content (0.847 and 0.465 respectively). The total number of genotypes was distributed into eight clusters (cluster I to VIII) in which cluster I comprised of highest number of genotypes (14) and contributed 31.11% to form the cluster. Iron content contributed maximum (64.14%) towards genetic divergence occupying first rank (635) towards divergence. From the variable factor map of first two principal components it was revealed that plant height was the most important morphological character for increasing the rhizome yield of turmeric.

Among the three group, low curcumin containing genotypes (Group I, 0.10 - 1.00%) had light yellow color of the rhizome whereas medium curcumin containing ones (Group II, 1.01-2.50%) exhibited light yellow to deep yellow color. High curcumin containing genotypes (Group III, 2.51-5.12%) possessed deep yellow to deep orange color of the rhizome. TCP 2 had the highest curcumin content (5.12%). Group II and III contained high total phenol and high iron containing genotypes whereas Group I contained the high crude fat containing genotypes (TCP 100 and TCP 221). Group III contained high antioxidant activity and high antioxidant enzyme activity containing genotypes. Curcumin itself exhibited maximum absorbance at 425 nm (1.170). But when pH was changed from acidic to alkaline, the absorbance value showed decreasing trend and colour changed from light yellow to brick red. Among three principal components, PC1 was the most important because almost all biochemical parameters were strongly and positively contributed. From the correlation table, it was observed that increase in curcumin content also increased the amount of iron, total phenol, total protein, catalase, peroxidase, superoxide dismutase, antioxidant activity and redness (a^* value) of the genotypes. The results revealed that majority of the biochemical parameters were found to be present in TCP 195 in highest or close to the highest quantity. So, this genotype was biochemically the best among the 45 genotype studied. But considering the yield, rhizome colour and most importantly the curcumin content of the genotype TCP 2, over TCP 195 this genotype may be recommended to the farmers for the commercial cultivation practices.

For standardization of protocol for micropropagation of *C. longa*, the genotype TCP 2 was taken as this cultivar shows promising yield and quality attribute. Along with TCP 2, black turmeric (*C. caesia*) another medicinally important endangered curcuma species was taken for mass multiplication. This curcuma species is an unexplored plant species but known for its ancient use in Ayurveda. Rhizome buds of TCP 2 (*C. longa*) exhibited best performance in shoot bud induction in MS medium containing 5.0 mg/L BAP + 1.0 mg/L NAA within 20 days while medium containing 5.0 mg/L BAP and 2.0 mg/L NAA showed proliferation of highest number of shoots/culture. *Curcuma caesia* rhizome buds showed best performance in 2.0 mg/L BAP + 0.75 mg/L IAA containing MS medium in shoot bud induction percentage within 18 days while MS medium with 2.0 mg/L BAP + 1.0 mg/L IAA (4.00) responded best in case of formation of number of shoots/explant (4.00). MS medium supplemented with a combination of 2.0 mg/L IBA gave the highest number of roots in both species while 2.0 mg/L 2,4-D showed the best result of callus induction for both explants. Among the two species, *C. caesia* showed better response in tissue culture with comparatively lower concentration of media components.

Introduction

Spices are the plant products those are used as food additives to supplement aroma and flavour in the foods. They contain various essential oils and other compounds which are responsible for their aroma and flavour. There are different types of spices, some of which can be used as medicines and some are used as food additives. Nearly 63 different spice crops are grown at various parts of India which is blessed with the diverse agro-climatic conditions those prevail in the country ranging from tropical to subtropical to temperate. Spices have very high demand both in the domestic and international market and therefore play an important role in the agricultural economy of the country.

One of the major spices grown in various parts of India is turmeric, *Curcuma longa* L., belonging to the family *Zingiberaceae*. Turmeric, a rhizomatous perennial herb and native of South East Asia, was first described by Carolus Linnaeus in the year 1753 in his book “*Species Plantarum*” (Ravindran *et al.*, 2007). The term *Curcuma* is derived from Arabic word “kurkum” which means “yellow” and the term *longa* refers to the elongated shape of its rhizome. It is also known as “Golden spice” (Ravindran *et al.*, 2007). According to Northern Territory Government (2010), the *Curcuma* species are widely distributed in the tropics of Asia ranging from India to South China, Papua New Guinea and also to northern Australia. India is the largest producer of turmeric in the world and contributes almost 80% of total production (Yanai, 1998) and 90% of world’s demand (Olojede *et al.*, 2009). About 92% of the total production of turmeric in India is consumed for domestic purpose and the remaining 8% is exported.

Curcuma longa L. (syn. *C. domestica* Val.), commonly known as turmeric or haldi (in Bengali and Hindi), is an important commercial crop of India, Sri Lanka, Pakistan, Bangladesh and China. Turmeric, a native of South East Asia, particularly of India, has served as one of the most ancient and important spices in the world. The rhizomes of turmeric are the cleaned, boiled, dried, polished and ground to powder

as processed product. It has wide applications in daily culinary activities in household kitchen to dyeing industry since time immemorial. It also has extensive application as condiment, flavouring and colouring agent. Turmeric has also been used as a remedy for many diseases. It has anti-cancer and anti-viral activities and hence finds use in the drug and cosmetic industry. The increasing demand for natural products as food additives makes turmeric an ideal choice as a food colorant. This multipurpose ancient and sacred spice of India, also known as '*Indian Saffron*', finds a place in offerings on religious and ceremonial occasions.

The terrain plains of North Bengal have the unique positional advantage of being in the juncture of three major phyto-geographical regions, namely the north-east, the eastern peninsula and the eastern Himalaya with thick natural forests. This makes this region very rich in diversified crop species. The terrain plain is surrounded by jungles and tea gardens from all the sides and consist of three districts namely Jalpaiguri, Alipurduar and Coochbehar. With the disadvantage of being the most distant districts from the state capital Kolkata, the region is fighting against low agricultural productivity and industrial backwardness and consequently affected with high unemployment and rural poverty. Turmeric is an ideal crop to substantiate the economy of this region as the terai agroclimate and soil conditions favour its cultivation. But in this region the local cultivars of turmeric are grown in small areas and the productivity is also very low.

Our university has a devoted research wing for improvement of turmeric. The local and introduced genotypes of turmeric, collected from the eastern and the north eastern geographical locations, are maintained under the aegis of All India Coordinated Research project (AICRP) on Spices, UBKV.

One of the major limitations for further improvement of the available cultivars is lack of information on their authentic phenotypic characters as well as biochemical profiling studies which can be utilized for proper scoring of the cultivars. Genotype collection represents the main source of variability for genetic improvement of turmeric and it is conducted mainly due to the economic importance of the crop and high genetic diversity observed (Lynrah *et al.*, 1998 and Singh *et al.*, 2003). The availability of information on estimates of heritability and genetic advance mean in respect of yield and yield determining traits were the basic

requirement for formulating the suitable breeding strategy in respect of genetic improvement program in turmeric (Maurya *et al.*, 2018).

Curcumin, a member of the family of natural polyphenol, is the major pigment present in rhizomes of turmeric. The primary flavoring components of volatile oil of turmeric include cineole and borneol (Guenther, 1952). These along with several other chemical components have made turmeric one of the world's most popular medicinal spices used in ethnic medicines (Ammon and Wahl, 1991). It is utilized for flavouring and colouring of a variety of dishes at domestic scale as well as in food industries. It has already gained importance all over the world as a potential component of therapeutic uses owing to its anti-inflammatory, antidiabetic, anticarcinogenic, antihepatotoxic, antiviral, choleraic, antibiotic, antivenomous and antirheumatic properties. It improves the complexion of body and is useful in diseases of blood, inflammations (Arora *et al.*, 1971), ozoena, dyspepsia (Thamlikitkul *et al.*, 1989), ulcer (Vandau *et al.*, 1998), elephantiasis, snake-bite, leucoderma, smallpox, sprains and urinary discharges and bruises (Kirtikar and Basu, 1996). The juice of rhizome is used as anthelmintic as well as in asthma and gonorrhea (Tiwari and Agrawal, 2003).

In addition to curcumin, the rhizomes of turmeric contain a wide array of secondary metabolites which impart its quality as well as medicinal property. A number of studies have revealed the presence of flavonoids (Kim *et al.*, 2011), tannins and anthocyanins (Niranjan *et al.*, 2003), vitamins such as thiamine, pyridoxine, niacin and riboflavin (Kubmarawa *et al.*, 2007), essential oils rich in terpenoids, short chain alcohols, ketones, and fatty acids (Braga *et al.*, 2003) and very good amounts of minerals like iron, calcium, zinc, potassium, magnesium, phosphorus and sodium (Mane *et al.*, 2018). The volatile constituents of turmeric contain bioactive compounds like turmerone, zingiberene, α -turmerone, curumene, cineole, p-cymene and curlone (Chainani-Wu, 2003). The curcuminoids and other phenolic compounds present in turmeric have been found to exhibit excellent antioxidant activities (Wojdylo *et al.*, 2007 and Mosovskaa *et al.*, 2016). Thus, in addition to curcumin, a number of bioactive compounds belonging to different chemical classes play important roles in the diverse biological activity of turmeric.

Turmeric is an herbaceous perennial with long, broad, lanceolate and bright green leaves. The flowers are pale yellow and borne on dense spikes. The

economically important part is the rhizome which becomes ready for harvest in about 7 to 9 months after planting. It is propagated exclusively through rhizomes. To plant one hectare of land, about 2000–2500 kg seed rhizomes (‘settes’) are required. ‘Mother rhizomes’ or ‘primary tubers’ (the swollen underground part at the base of aerial shoots) as well as larger side rhizomes (3–6 cm long, having two to three good eyes) are the commonly used planting materials. Conventionally larger sized-rhizomes are believed to be the best planting material for vigorous plant growth and higher yields. For sowing, both the mother-rhizomes as well as fingers are used. The fingers are cut into 4-5 cm long pieces and the mother rhizomes are planted as such or split into two; each having at least one sound bud.

By the traditional propagation method the maximum multiplication that can be achieved annually has been observed to be 5–10 fold which is not profitable. Moreover, the conventional method of propagation has three major disadvantages:

- A portion of 10–20% of the yield has to be set aside for raising the next crop which reduces the income of the farmers;
- Transmission of soil borne diseases from one crop to the next, and from one location to another imposes serious restriction on the export quality of the harvest;
- After the harvest of the crop and planting for the next season the seed rhizomes need proper preservation as they are prone to rhizome rot caused by bacteria and fungi, and storage insect attacks.

Furthermore, turmeric cannot be improved by breeding approaches because flowering is rare and often seed set does not occur. The only method available for the improvement of the crop is through selection of desirable genotypes from natural population or generation of new genotype by somaclonal variation through tissue culture approaches. The desirable criteria for the improvement of turmeric are based on higher yield, higher curcumin content, disease and insect-pest resistance, better adaptability, etc. Nowadays, *in vitro* cell and tissue culture methodology is widely used to ensure the survival of endangered plant species and rapid mass propagation for large-scale cultivation. Various protocols are available for micropropagation of turmeric varieties. These micropropagation techniques for the rapid clonal multiplication of turmeric species are widely used for conservation of genotypes and

production of microrhizomes *in vitro*. Tissue culture generated microrhizomes have many advantages as compared to traditional cultivation system. It helps to save a large amount of rhizomes from the previous harvest which are used to serve as seed material for the next cultivation. The microrhizomes generated through tissue culture is disease free, so the risk of crop loss due to soil and seed borne pathogens are less. The unavailability of elite variety seed material is an obstruction for quality cultivation and high economic output. Tissue culture is a perfect solution to this problem as many plantlets and microrhizomes can be produced from small amount of explants. Hence, establishment of micropropagation technique for elite and region specific turmeric species can solve the problem of scarcity of quality planting materials of turmeric.

The genus *Curcuma* has a number of species of which black turmeric (*Curcuma caesia* Roxb.) is medicinally important one. This species is native to North-East and Central India. It is a perennial herb with bluish-black rhizome. The leaves of *Curcuma caesia* have a deep red-violet patch, which runs throughout the whole lamina. The rhizome has profound economic importance due to its medicinal properties, claimed to be useful in treating several disease like leprosy, bronchitis, asthma, piles, cancer, epilepsy, fever wounds, impotency, fertility, tooth ache and vomiting etc. (Flowers of India, 2005). Presently this species is said to be under threat of extinction since natural habitat is being destroyed widely through several human activities like industrialization, urbanization etc. So it is necessary to take some measures for conservation of this unique species.

In spite of having a large number of turmeric genotypes grown in this northern region of West Bengal, these have not been scored properly based on their phenotypic and biochemical characters. Information regarding detailed biochemical and nutritional composition as well as their enzymatic and antioxidant activities of these genotypes is scarce. To fill up this gap and also to standardize protocol for tissue culture of the best genotype from the studied ones suitable to grow in this region, the present programme entitled “Biochemical characterization of turmeric (*Curcuma longa* L.) germplasms and standardization of protocol for micropropagation of selected cultivars” has been taken up with the following three objectives:

- 1) Scoring of collected germplasms of turmeric based on statistical analysis of phenotypic and biochemical characters.
- 2) Estimation of quality related biochemical parameters of some selected cultivars/varieties and attempt to find correlation among different parameters.
- 3) Standardization of protocol for micropropagation of best of the selected cultivars.

Under the first objective scoring of the collected genotypes has been performed by recording data of seven phenotypic characters namely plant height, number of tillers per plant, number of leaves per plant, length of leaf, leaf breadth, rhizome weight and yield and two biochemical parameters namely curcumin content and iron content followed by statistical analysis of the recorded data.

In case of second objective the biochemical parameters such as estimation of curcumin, protein, total phenol, crude fat and iron; assay of activity of antioxidative enzymes namely catalase, peroxidase and superoxide dismutase; assay of antioxidant activity by ABTS, DPPH, TAC and CUPRAC methods; rhizome colour and measurement of pH changes of rhizome powder has been measured.

The third objective deals with standardization of protocol for tissue culture of the genotype found to be best from the earlier studies. The micropropagation technique for black turmeric (*Curcuma caesia* Roxb.) has also been standardized under this objective.

Review of Literature

Considering the objectives of the present programme, this chapter reviews the works done earlier with regards to the scoring of different germplasms of turmeric, analysis of their biochemical parameters and micropropagation strategies adopted by various workers.

2.1 General review on turmeric

2.1.1 Origin

Turmeric (*Curcuma longa* L.) is native of sub-Himalayan mountain region and grown widely in many parts of the tropical and subtropical regions as an important commercial crop. Parthasarthy *et al.* (2007) reported that turmeric ($2n = 3x = 63$) originated in South-East Asia.

2.1.2 Botany

Turmeric is a perennial herbaceous plant. The plant reaches up to 1 m height. It produces highly branched and cylindrical, yellow to orange coloured rhizomes. Leaves are of alternate type and arranged in two rows. They are sub-divided into leaf sheath, petiole and leaf blade. A false stem is formed from the leaf sheaths. The petiole is 50 to 115 cm long while the simple leaf blades are usually 76 to 115 cm long. They have a width of 38 to 45 cm and are oblong to elliptic, narrowing at the tip (Grieve, 2017).

2.1.3 Cultivation

Turmeric is a tropical crop cultivated from almost at sea level to 1500 meter above mean sea level with moderate rainfall. It thrives best in well-drained, fertile, sandy and black, red or alluvial loam soil with a pH range of 4.5-7.5 having good organic matter content. Turmeric can be grown in diverse tropical conditions at a temperature range of 20-35°C with an annual rainfall of 1500 mm or more, under rainfed or irrigated conditions. Turmeric is sown in the month of May and harvested in the last week of January or first week of February. The rhizome of this plant, also

referred to as root, is the most useful part of the plant for culinary and medicinal purposes. The price of turmeric is directly influenced by the content of the pigment curcumin (Anon., 2007).

2.2 Scoring of turmeric germplasms

Different workers have carried out experiments to compare the variability and association among various phenotypic and genotypic characters and also the heritability of them. A brief review of the works related to the above aspects is being described below.

One hundred genotypes were collected in Andhra Pradesh within the country and broadly classified into three duration groups depending on their crop life. These were 1) Short duration "Kasthuri" types belonging to *C. aromatica* maturing in 7 months referred as "Ca" types, 2) Medium duration "Kesari" types and referred to as intermediate or CLI types. 3) Long duration types belonging to *C. Zanga* maturing in 9 months and are referred as CLL types. Clonal selections were made among the more popular cultivars in these duration groups and yield trials carried out for three years resulted in identification of eight promising types in short duration, six types in medium duration and eight in long duration group (Rao *et al.*, 1975).

Thirteen varieties of turmeric were taken in an experiment to study the phenotypic and genotypic coefficient of variation and heritability of different characters. It was observed that the phenotypic coefficient of variation was in general higher than genotypic coefficient of variation. The genotypic coefficient of variation was very high for weight of root tubers followed by rhizome yield, leaf area, number of primary fingers and number of tillers indicating high degree of genetic variability for these characters. High heritability in broad sense was also found for weight of root tubers, rhizome yield, crop duration, number of leaves and number of primary fingers (Reddy and Rao, 1988).

In another study yield and eleven of its related characters in ten turmeric genotypes were evaluated. All the characters except weight of mother rhizome and yields of primary and secondary fingers per plant showed high heritability. Number of secondary fingers per plant was the only character that showed a significant and positive correlation with turmeric yield (Jalgoankar and Jamdagni, 1989).

An experiment was conducted for evaluation of turmeric genotypes and it was observed that exploitation of genetic resources in turmeric had resulted in the identification of a few breeding stocks and promising genotypes. Yield of fresh rhizome/clump, weight of primary finger and number of primary fingers/clump exhibited very high degree of variability (Chandra *et al.*, 1998).

Twenty-five turmeric genotypes were studied for variability and association of characters for three consecutive years. Among the 19 characters studied, weight of primary finger rhizome recorded the highest level of variability (38.94%) followed by number of primary and secondary finger rhizome per clump. Plant height, length of leaf, weight of primary finger rhizome, internode distance of primary finger rhizome and rhizome yield per hectare were significantly and positively correlated with fresh rhizome yield per clump. A negative association between dry rhizome recovery and fresh rhizome yield per clump was observed. The varieties PCT 13, PCT 11, GL Puram and PCT 15 among the studied ones showed no significant difference and had higher yields, indicating their suitability for cultivation under mid-hill conditions of Meghalaya. The variety Lakadong had poor yield but higher (7.33%) curcumin content (Chandra *et al.*, 1999).

Sixteen varieties of turmeric were compared in a study to identify the best one by considering the parameters like plant height, number of tillers per plant, number of leaves per plant, length and breadth of leaf, average weight of rhizome per plant and yield of rhizome. The variety DKHT-6 exhibited maximum value for all the above parameters mentioned (Singh *et al.*, 2000).

The genetic variability in the morphological and yield attributing traits of eleven turmeric germplasms (PTS-62, Kasturi, Sanjule Local, PTS-8, Roma, Rartga, PTS-14, PTS-55, Armoor, PET-14, and Nadia Local) was evaluated using mother rhizome as the planting material. No consistent variation in morphological traits was observed among the genotypes. Roma recorded the highest value for number of tillers and leaves per plant, Kasturi for plant height, Armoor for plant girth, PTS-8 for leaf length and PTS-14 for leaf breadth. Roma also produced the highest number of mother rhizomes and primary and secondary fingers whereas Kasturi and PTS-62 recorded the highest length and weight of mother rhizome, primary and secondary fingers respectively. Kasturi produced the highest yield (54.68 t/ha) followed by PTS-62 (49.58 t/ha), Armoor (46.47 t/ha) and PTS-8 (44.48 t/ha) (Datta *et al.*, 2001).

Fifteen turmeric cultivars were studied to identify the best suited high yielding cultivars for the terai region of West Bengal, India. Mean dry rhizome yield was highest in PTS-62 (3.31 kg/3m² plot) followed by ACC-360, Nagaland Local and ACC-361. On the basis of higher dry rhizome yield, PTS-62 was found to be the best among the turmeric cultivars tested followed by ACC-360 (Panja *et al.*, 2001).

A study was carried out to evaluate the performance of twenty-three turmeric cultivars and identify the best one suited for cultivation in Manipur, India. Meghalaya Local was the tallest (233.86 cm) followed by Duggirala-325 (133.46 cm) and PCT-II (130.63 cm). The number of tillers per plant varied from 3.38 in Duggirala-325 to 2.20 in both Chayapasupa and Dehradun Local. The rhizome length ranged from 9.41 cm in Thoubal Local to 6.06 cm in Nagaland Local. Rhizome girth was maximum in Thoubal local (21.00 mm) and lowest in Sikkim Red (13.66 mm). PCT-8 and Sikkim Yellow had the maximum (21.8 cm) and minimum (13.2 cm) primary finger lengths respectively. The fresh yield of rhizome was highest in Thoubal Local (31.78 t/ha) and lowest in Sikkim Red (17.00 t/ha). Kasturi Tanaka had the maximum dry matter recovery percentage (21.20%) while Sikkim Yellow had the minimum (16.86%). In terms of total dry yield, Duggirala-325, Thoubal Local and GL-Puram were the best for this condition. Therefore, it was concluded that these genotypes might be used for large scale cultivation of turmeric in the state (Kumar and Yadav, 2001).

Nineteen characters of eight turmeric genotypes were evaluated. It was revealed that plant height, number of leaves, number of primary fingers, number and weight of secondary fingers had positive genotypic correlation while length of primary finger had significant negative correlation with rhizome yield. The number and weight of secondary rhizome exhibited higher positive correlation with yield than the other characters. Path analysis indicated higher positive effect of weight of primary and mother rhizomes but non-significant correlation with rhizome yield due to higher negative effect of some of the characters. The number of primary fingers, on the contrary, had the highest direct negative effect on rhizome yield but significantly positive correlation with it. Plant height, number of leaves, number and weight of secondary fingers and number of primary fingers were the important traits for selection of better turmeric genotypes (Panja *et al.*, 2002).

Genetic variation and heritability for rhizome yield and yield components in 65 turmeric genotypes were studied. The greatest variation was found for yield followed by weight of mother rhizome per plant, plant height, weight of primary rhizome per plant and number of leaves. Phenotypic coefficient of variation was higher than genotypic coefficient of variation for all the characters. High values of heritability and genetic gain were recorded for all the traits except number of mother rhizome per plant which exhibited low magnitudes of heritability and genetic gain. The results suggest that superior genotypes might be obtained through selection based on the number and weight of mother, primary and secondary rhizome (Singh *et al.*, 2003).

Ten genotypes of turmeric were studied to select the most suitable cultivar for growing as inter-crop in an arecanut plantation. The parameters examined were plant height, leaf number, leaf length, leaf breadth, the number, weight, length and breadth of primary and secondary fingers, the weight, length and breadth of clumps, yield per plot and projected yield per hectare. PTS-43, Suguna, ACC-360 and Roma were the most suitable ones among the studied germplasms for cultivation as inter-crop in the alluvial plains of West Bengal and the inter-crop had no adverse effect on the growth of the main crop (Bandyopadhyay *et al.*, 2003).

Histological and morphological difference was studied in the leaves of *Curcuma* based on optical microscope and electronic microscopic scanning. There were no distinct histological and morphological differences among the leaves of turmeric (Xiao *et al.*, 2004).

As revealed in this study, the characters like number of tillers, plant height and weights of primary, secondary and fresh rhizome of turmeric could be utilized for character association and path analysis for yield components. Plant height, leaf length and thickness of primary and secondary rhizomes exhibited positive direct effect on the yield of turmeric rhizome (Tomar *et al.*, 2005).

Forty-one turmeric genotypes were evaluated for twelve characters, i.e. yield per plant, plant height, clumps per plant, leaves per plant, primary and secondary rhizomes per plant, length of mother, primary and secondary rhizomes and weight of mother, primary and secondary rhizomes. All the characters showed positive correlation with yield at both the phenotypic and genotypic level. However length

and weight of mother and primary rhizome had the highest significant correlation with yield. Weight of mother, primary and secondary rhizomes had the highest positive direct effects on yield. They concluded that characters showing high correlation and direct effect on yield may be exploited in turmeric improvement programmes (Yadav *et al.*, 2006).

The growth and yield parameters of eleven turmeric varieties were studied in which the variety Megha showed highest plant height (135.17 cm) followed by Rasmi (117.89 cm) and Duggirala Red showed the lowest height (88.49 cm). Highest tiller number was exhibited by the genotype IISR Pratibha (3.20) whereas lowest was recorded in Rasmi (2.20). BSR-2 and TCP-2 recorded the highest number of leaves (11.13). TCP-2 exhibited the highest clump weight (373.20 g) followed by IISR Kedaram (354.70 g). Highest rhizome yield per plot was found in Narendra Haldi-1 (13 kg) followed by TCP-2 (12 kg) and the lowest was observed in Duggirala Red (6 kg) (Anon., 2010).

The qualitative characters like colour of leaf, rhizome and flower and quantitative characters including plant height, leaf length, bract width, spike length, spike width, peduncle length, ligule length, rhizome length, rhizome width, rhizome branches number, rhizome nodes number and corolla tube length were taken into consideration for characterization of twenty turmeric germplasms. The result revealed that 20 genotypes were subdivided into two main clusters with three sub-groups in the 2nd cluster and evaluated genotypes of turmeric germplasm appeared to have narrow genetic base (Hikmat *et al.*, 2012).

In an experiment, eighty turmeric genotypes were studied in a randomized block design to study the variability, heritability with genetic advance, correlation, direct and indirect effects of characters on rhizome yield for a period of four year. Heritability and genetic advance were high for rhizome yield and number of tillers per plant. Genotypic correlation was observed to be higher than phenotypic correlation for all the characters under consideration indicating poor influence of environment and presence of inherent association between various characters. High estimates of heritability were recorded for the number of tillers per plant indicating the contribution of a major part of the phenotypic variability by additive gene effects. Number of tillers/plant followed by plant height exerted the highest positive direct effect on rhizome yield which indicated that selection for these characters would

directly improve the yield in turmeric. It was also reported that the yield per plant was positively significant with the number of leaves per plant (Rajyalakshmi *et al.*, 2013).

Now-a-days, the use of phenotypic characters in genotype characterization is going to be limited due to availability of small number of descriptors and also due to the influence of environment and interaction of genotype with environment (Ferreira *et al.*, 2007). Therefore, researchers are using RAPD (Random amplification of polymorphic DNA) and SSR (Simple sequence repeats) markers to ascertain the genetic variation among the genotypes of various crop plants at DNA level. Molecular marker techniques may also control many limitations of the morphological and biochemical markers for the distinction of plant genotypes by providing genetic background for the recorded phenotypic variability since they are not influenced by the environment or developmental stage and can distinguish the variation at the DNA level (Jan *et al.*, 2012).

In such an experiment the genetic diversity among fifty seven turmeric accessions collected from Brazil, India and Puerto Rico were examined. From lyophilized leaf tissue total DNA was extracted and genetic analysis was carried out using seventeen microsatellite markers (SSR). Analyses of molecular variance showed major differences (63.4%) among the countries and highest genetic diversity (75.3%) within the states of Brazil (Sigrist *et al.*, 2011).

Directed Amplification of Minisatellite DNA (DAMD) and Inter Simple Sequence Repeats (ISSR) methods were employed to estimate the genetic variability in 29 genotypes of turmeric of India in a separate study. Data analysis for DAMD (15) and ISSR (13) markers revealed 82% polymorphism among the genotypes and broad range of pairwise genetic distances (0.03–0.59) established wide genetic diverseness among them. The results establish the efficiency and reliability of DAMD and ISSR markers in determining the genetic diversity and relationships among the turmeric germplasms (Verma *et al.*, 2015).

In another study, eighty three turmeric (*Curcuma longa* L.) genotypes were evaluated to estimate the genetic diversity and relationship among the genotypes using morphological and molecular markers. Among the twenty one RAPD primers, all except OPC (Oligonucleotide purification cartridge) 16 exhibited the PIC

(Polymorphism information content) value more than 0.5 with the product sizes ranging from 190 bp to 1500 bp. On the other hand, all the SSR primers produced distinct, scorable and clear bands. The PCR product size acquired by the amplification of SSR primers varied from 110 bp to 440 bp with a total of 38 fragments. All the SSR markers used in the genetic analysis were polymorphic. The markers were observed to be reliable to recognize the duplicates in the genotypes whereas RAPD markers could identify closely related ones. Among the 83 genotypes studied, nine sets of synonymous entities were identified, indicating that SSRs are more accurate and reliable than RAPDs to study the genetic diversity in turmeric (Mamatha, 2016).

Another study was carried out for identification and assessment of eight genotypes of turmeric collected from all ecological zones in India by RAPD markers. After isolation of DNA and performing PCR, data analysis showed different levels of genetic polymorphism by most of the RAPD markers. It was concluded that *C. longa* undergoes genetic variation due to a wide range of ecological conditions within distribution area (Ashraf *et al.*, 2017).

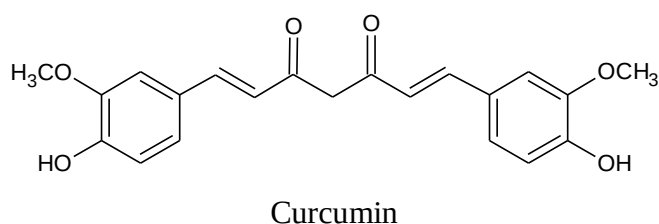
2.3 Quality related biochemical parameters

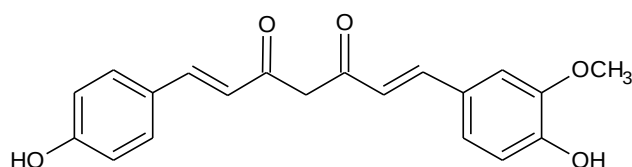
A brief review of the previous works related to the biochemical composition and curcumin content of different turmeric varieties are described below.

2.3.1 Biochemical composition of turmeric

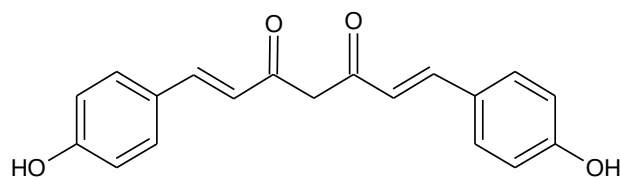
The principal coloring constituent of *Curcuma longa* is curcumin (diferuloyl methane) and two of its closely related compounds namely demethoxycurcumin (*p*-coumarylferuloyl methane) and bisdemethoxycurcumin (di-*p*-coumarylmethane). These three together are called curcuminoid, and are present in turmeric rhizomes approximately in a ratio of 3.5:2:1 (Ammon and Wahl, 1991).

The chemical structures of the three components of curcuminoids are as presented below:





Demethoxycurcumin



Bis-demethoxycurcumin

The composition of curcuminoids in turmeric rhizome was estimated to be approximately 70% curcumin, 17% demethoxycurcumin, 3% *bisdemethoxycurcumin* (Aggarwal *et al.*, 2007). The most bioactive component of turmeric among these curcuminoids was curcumin which made up 2 to 5% of the spice (Sandur *et al.*, 2007).

A number of spectrophotometric methods were reported for the quantification of the curcuminoids. The detection wavelengths were set at 420-430 nm at which curcuminoids have their maximal absorption (Ruby, 1995; Braga, 2003; Manzan, 2003).

The amount of curcumin in turmeric depended on various factors such as storage conditions, microbial attacks etc. The curcumin content varied from fresh to stored rhizomes (3.43 to 5.78%). It increased up to two and half years when estimated at six months interval but after three years it decreased (3.19%) due to fungal infection, surroundings of storage etc. in comparison to fresh rhizomes (3.426%) (Ganapati *et al.*, 2011).

In a study to compare colour with curcumin content it was observed that visual colour difference of five turmeric varieties (Indian I, Indian II, Local I, Local II and Local III) ranged from bright yellow to orange yellow color and curcumin content varied from 3.76 to 5.05% (Madhusankha *et al.*, 2018).

The variation in the rhizome essential oil along with its quality and curcumin contents in twenty-seven land races of *C. longa* of North Indian plains were studied. It was observed that the curcumin content varied from 0.61% to 1.45% whereas oil

content of rhizomes differed from 0.16% to 1.94% on a fresh weight basis (Garg *et al.*, 1999).

Essential oil of turmeric was assayed by different workers and was found to contain nearly 100 compounds. These were terpenes, oxygen derivative terpenoids, short chain alcohols, ketones and fatty acids (Raina *et al.*, 2002; Jayaprakasha *et al.*, 2001; Garg *et al.*, 2002; Braga *et al.*, 2003).

The essential oil content was estimated in six varieties of turmeric and was found to vary from 3.75% in Pratibha to 7.00% in IET RH-17, RH-401, RH-406, RH-410 and RH-411 (Anon, 2011).

The rhizomes of fourteen varieties of turmeric were analyzed for curcumin and essential oil content. The curcumin content in the dried rhizomes ranged from 0.58-2.18% while essential oil content varied from 0.05-1.40%. The variety Rajendra Sonia contained the highest curcumin (2.18%) whereas Suvarna had maximum essential oil (1.40%) among the fourteen varieties (Rai *et al.*, 2016).

Extraction of essential oil and pigments from *Curcuma longa* was done by steam distillation with volatile solvents. In this experiment, the best processing condition was developed to maximize the yields of essential oil and pigments. Autoclave pressure and distillation time were the variables studied for the steam distillation process. The highest yields of essential oil (0.46% by weight) and pigment (0.16% by weight) expressed as curcumin, demethoxycurcumin and bisdemethoxycurcumin were obtained at a pressure of 1.0×10^5 Pa and a time of 2 hours (Manzan *et al.*, 2003).

In a study to estimate the volatile oil and curcumin content in turmeric rhizomes the range of volatile oil content was found to vary from 2.5-7.2% and curcumin content from 1.8-5.4% (Krishnamurthy *et al.*, 1975).

The biochemical compositions of turmeric finger rhizomes were assayed in which moisture was in the range of 7.01 to 7.91%, ash 4.34 to 5.07%, crude protein 4.05 to 7.09%, crude fat 2.66 to 4.46%, crude fiber 2.09 to 3.67% and carbohydrates 73.47 to 78.31% respectively (Fattepurkar *et al.*, 2009).

Curcuminoids are natural phenols. The phenolic content of turmeric was estimated in a study and was observed to be 6.3 mg GAE/g dry weight (Shan *et al.*, 2005).

The total phenolic content and total flavonoid content of thirteen commonly used spices were estimated. Among the thirteen spices, total phenolic content and total flavonoid content of turmeric extract were 10.17 µg CE/g and 101.34 µg QE/g respectively (Kim *et al.*, 2011).

Dried rhizomes of *C. longa*, fresh rhizomes of *C. longa*, *C. amada* and *C. zeodaria* as well as leaves, petioles and stems of all species were analyzed to study the biochemical contents. In the rhizomes, protein content was 3.6-6.8%, anthocyanins 18.9-37.0 µg/g, phenols 0.15-0.62%, tannins 0.32-0.76%, sugars 20.5-43.4%, essential oil 3.7-5.3%, curcumin 3.4%, ash 6.9-9.8%, moisture 90.2-91.3% and ethanol and ethanol:water extractives 7.5-13.4% and 10.5-19.5% respectively (Niranjan *et al.*, 2003).

Phytochemical analysis of two medicinally and industrially important *Curcuma* species i.e. *Curcuma aromatica* and *Curcuma xanthorrhiza* revealed the presence of phytochemicals like flavonoids, tannin, saponin, terpenoids in both *Curcuma* species. The amount of curcumin was higher in *C. xanthorrhiza* (1.086 g /100 g) as compared to *C. aromatica* (0.0175 g /100 g) (Anjusha and Gangaprasad, 2014).

Turmeric is a very rich source of many essential vitamins such as pyridoxine (vitamin B₆), niacin and riboflavin and also contains very good amounts of minerals like calcium, iron, potassium, manganese, copper, zinc, and magnesium. One hundred gram herb provided 1.80 mg of daily recommended levels of pyridoxine. Fresh root contained very good levels of vitamin C. One hundred gram of root consisted of 23.9 mg of this vitamin (website: www.nutrition-and-you.com).

An experiment was carried out to study the curcumin percentage from Salem, Krishna, Rajapuri and Pratibbha varieties and these varieties were extracted by ethanol, acetone, hexane, and HPLC (high performance liquid chromatography) method. It was found that the highest range of curcumin percentage was observed from 3.584 to 7.730% in the variety Pratibbha followed by Salem 2.169 to 5.932%, Rajapuri 2.812 to 4.366% and Krishna 1.599 to 3.520%, respectively. The highest percentage of curcumin was extracted by HPLC method. The range of curcumin extracted by HPLC method was found to be 2.308 to 5.662% from boiled turmeric and 3.52 to 5.93% from steam cooked turmeric, respectively (Kamble *et al.*, 2011).

Curcumin and essential oil contents were estimated in some turmeric (*Curcuma longa* L.) cultivars of Maharashtra and it was found that the curcumin contents in mother rhizomes of all cultivars were comparatively higher than those in the fingers, except in the case of cv. Rajapuri. Mother rhizomes of Mydukur and fingers of Salem showed the highest curcumin content (Rakhunde *et al.*, 1998).

The turmeric variety Alleppy showed highest curcumin content (6.2%) among the selected 20 cultivars (Muralidharan and Ramankutty, 1976).

The variations in curcumin content in *Curcuma longa* germplasms namely Rashmi, Krishna, Roma, CL-315, CEL-6, CL-70, CL-13 and CL-16 collected from various locations of India were studied and Rashmi was found to have highest curcumin (Dixit *et al.*, 2002).

Among the turmeric genotypes of different growing regions in Meghalaya, Lakadong variety was reported to have the highest (6.8%-7.3%) curcumin content (Kanjilal *et al.*, 2002).

In a study to assess the levels of macro, micro and toxic heavy metals in some branded and unbranded turmeric powders available in Sri Lankan market, it was observed that iron content of turmeric samples ranged between 203.6-461.7 µg/g which exceeded the maximum limit recommended by WHO (Withanage *et al.*, 2015).

The nutritional and physicochemical properties of fresh turmeric rhizome were analyzed in another study in which the colour of rhizome was observed to vary from yellowish brown to deep brown. The rhizome contained 84.25% moisture, 9.10% carbohydrate, 1.20% protein, 1.08% fat, 5.1% curcumin, 0.66% ash, 0.72% fiber, 0.70% acidity and pH 5.7. The mineral content of fresh rhizome showed 22.9 mg/100g zinc, 8.2 mg/100g calcium, 2.3 mg/100g iron, 1.70 mg/100g potassium, 0.19 mg/100g magnesium, 0.12 mg/100g phosphorus and 0.06 mg/100g sodium (Mane *et al.*, 2018).

In one experiment the proximate analysis and mineral composition of *Curcuma longa* rhizomes were studied where 76.02% moisture, 16.37% carbohydrate, 3.04% ash, 1.95% crude fibre, 1.83% proteins, and 0.80% fat were found to be present. The mineral content were as follows: iron (0.57%), potassium (0.42%), magnesium (0.05%), phosphorus (0.03%), calcium (0.02%) and sodium

(0.01%). It was also found the rhizome to be rich in steroids, anthraquinones and terpenes (Ahaotu and Lawal, 2019).

2.3.2 Antioxidant and enzymatic activity of turmeric

Antioxidants are substances that inhibit the oxidation of other compounds. Antioxidants include enzymes, vitamins, thiols, polyphenols and other compounds. Natural antioxidants like vitamin E, phenolics, ascorbic acid and rosemary extract found in higher plants are used in food industry as food additives to help prolong shelf life and appearance of many food commodities. They can protect the human body from deleterious effects of the free radicals (Trombino *et al.*, 2004). Antioxidant compounds play an important role in prevention of diseases induced by free radicals by scavenging them (Yamaguchi *et al.*, 1998).

Curcumin is a unique antioxidant, which contains a variety of functional groups (Jovanovic *et al.*, 1999). Phenolic groups are responsible for radical scavenging properties of the specific antioxidant reactants. The clinical use of curcumin was limited because of its low bioavailability due to the hydrophobic nature of the molecule (Aggarwal *et al.*, 2007).

Oxidative damage is believed to be one of the mechanisms behind aging and many diseases. It involves free radicals, highly reactive species with unpaired electrons. Free radicals tend to react with important organic substances, such as fatty acids, proteins or DNA. Free radicals are constantly formed in living cells and removed by antioxidant defenses. Antioxidant enzymes are the main line of defense against free radicals in animal and plant cells. The primary antioxidant enzymes are superoxide dismutase (SOD), catalase and peroxidases. SOD catalyzes the simultaneous oxidation and reduction of the superoxide radical to produce oxygen and hydrogen peroxide whereas catalase catalyzes the conversion of hydrogen peroxide to produce water and oxygen. Peroxidase is an enzyme that catalyzes the oxidation of a particular substrate by hydrogen peroxide. The DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity are two most widely used methods for estimation of antioxidant activity of phytochemicals (Sharma and Bhat, 2009).

The antioxidant activity of thirteen turmeric species were estimated and was found to be 24.43% DPPH activity and 68.09% hydroxyl radical scavenging activity respectively (Kim *et al.*, 2011).

The curcuminoids inhibited generation of potent free radicals like superoxide and hydroxyl radicals (Reddy and Lokesh, 1992). Tetrahydrocurcumin had the strongest antioxidant activity among the various curcuminoids (Sugiyama *et al.*, 1996). Turmeric containing active constituent curcumin showed high antioxidant activity to inhibit lipid peroxidation & cyclooxygenase activity (Hussain, 2002).

Turmeric is a good source of bioactive compounds like antioxidant, polyphenols and flavonoids, which may be the substitute of antibiotics used in food and food products. Turmerone, zingiberene, ar-turmerone, curumene, cineole, p-cymene and curloneare are included in volatile constituents of turmeric while the nonvolatile constituents are curcuminoids (Chainani-Wu, 2003).

Curcumin was significantly more effective than many other spices for prevention of lipid peroxidation. Its antioxidant activity was eight times more effective than vitamin E and it was found to be more effective than synthetic antioxidants like butylatedhydroxytoluene (BHT) in preventing lipid peroxidation (Reddy and Lokesh, 1992).

Aqueous and ethanolic extracts of two different genotypes of turmeric from Bangladesh were used to assess the antioxidant properties and to quantify the polyphenol, flavonoid, tannin and ascorbic acid contents. The antioxidant activities were measured by DPPH and FRAP methods. The ethanolic extract of Chittagong's Mura variety had the highest amount of polyphenols (16.07%), flavonoids (9.66%), and ascorbic acid (0.09 mg/100 g). The ethanolic extract of Khulna's Mura variety exhibited higher DPPH radical scavenging activity (1.08 μ g/mL) whereas Khulna's Chora variety contained higher FRAP value (4204.46 $\pm$ 74.48 μ M Fe [II] per 100 g). Overall, ethanolic extract was found to have the higher antioxidant activity than the aqueous extract (Tanvir *et al.*, 2017).

Total antioxidant capacities of thirty two turmeric species were analyzed by ABTS (2,2-azinobis-3-ethylbenzthiazoline-6-sulfonic acid), DPPH (1,1-diphenyl-2-picrylhydrazyl radical) and ferric reducing antioxidant power (FRAP) method and

were recorded to be 19.5 μM Trolox/100 g (ABTS), 100 μM Trolox/100 g (DPPH) and 62.6 μM Trolox/100 g (FRAP) respectively (Wojdylo *et al.*, 2007).

A study was carried out to examine the isolation of curcuminoids from the dried turmeric rhizome and their antioxidant activity. The antioxidant activity was analyzed by two methods, such as ABTS and FRAP assay. The ABTS radical scavenging activity of curcuminoids was 163.4 $\mu\text{g/ml}$ whereas FRAP value of curcuminoids was 32.6 $\mu\text{g/ml}$. The existence of curcuminoids was also identified by Nuclear Magnetic Resonance (NMR) analysis (Mosovskaa *et al.*, 2016).

2.3.3 Biological and medicinal effects of turmeric

From long past turmeric has been showing profound importance in medical field by exhibiting anti-inflammatory, antimutagen, anticancer, antibacterial, antifungal, antiparasitic and detox properties possibly due to presence of curcumin and volatile oils in turmeric rhizomes. These constituents also promote liver and kidney functions, alleviate biliary disorders, and also diabetic and hepatic disorders (Hermann and Martin, 1991; Osawa *et al.*, 1995; Sugiyama *et al.*, 1996; Nakamura *et al.*, 1998).

Ethanol extract of turmeric showed significant decrease in ulcer index and acidity of stomach contents. Pretreatment with the turmeric extract lowered the intensity of ulceration. Hypothermic restraint stress reduction of gastric wall mucus was prevented by treatment of ethanolic extract of turmeric and decreased the severity of lesions induced by various necrotizing agents (Rafatullah *et al.*, 1990).

Curcumin has positive effect in maintaining the level of cholesterol and lipid peroxides. Oral intake of 500 mg/day curcumin for 7 days resulted in a significant increase in HDL cholesterol (29%) while 33% decrease in the level of serum lipid peroxides and 12% in total serum cholesterol (Soni *et al.*, 1992).

Curcumin functions as a promising chemo preventive agent by inhibiting the enzyme cytochrome P₄₅₀ A1 and thereby preventing the chemically induced toxicity (Commandeur and Vermeulen, 1996).

The anticarcinogenic property exhibited by curcuminoids is due to their oxygen radical scavenging activity (Soudamini and Kuttan, 1989). The induction of apoptosis plays an important role in its anticarcinogenic effect (Chen and Huang, 1998).

Turmeric influences apoptosis and inhibits cell cycle progression. It blocks cancerous cell growth in rat aortic smooth muscle cells (Gautam *et al.*, 1998).

Curcuma longa extract and its essential oil prevented the growth of different bacteria, parasites and pathogenic fungi. A study of chicks affected with the caecal parasite *Eimera maxima* revealed that diets companioned with turmeric resulted in reduction of small intestinal lesion scores and increased weight gain (Allen *et al.*, 1998).

Turmeric may be taken for the remedy of boils, bruises, blistering, ulcers, eczema, parasitic infections, hemorrhages and skin diseases (World Health Organization, 1999).

The protective characteristics of turmeric shown on the cardiovascular system include decreasing cholesterol and triglyceride levels, lowering susceptibility of low density lipoprotein (LDL) to lipid peroxidation and preventing platelet aggregation in incorporation to lower plasma cholesterol and triglyceride levels (Ramirez *et al.*, 1999).

Curcumin inhibited the growth of *Helicobacter pylori* Cag A+ strains *in vitro*. Curcumin and essential oil fraction prevented the growth of several bacteria like *Lactobacillus*, *Streptococcus* etc. (Kumar *et al.*, 2001).

Curcumin exhibited anti-HIV activity by preventing the HIV-1 integrase needed for viral replication. It also prevented HIV gene expression influenced by UV light. For this reason, curcumin can be helpful for novel drug establishment against HIV (Araujo and Leon, 2001).

The anticancer effect of curcumin is mainly mediated through induction of apoptosis. The anti-inflammatory, anticancer and antioxidant roles of turmeric can be clinically exploited to control rheumatism, carcinogenesis and oxidative stress-related pathogenesis. Curcumin has been used to reduce post-operative inflammation (Srimal, 1997; Das and Das, 2002).

Curcumin prevented the activity of certain chemotherapy drugs. Research showed that curcumin decreased camptothecin induced death of cultured breast cancer cells and inhibited cyclophosphamide-induced breast tumor regression in mice (Sakano and Kawanishi, 2002).

Curcumin could interfere in the intake and efficacy of the chemotherapy drug irinotecan used to treat colon cancer. In a mouse xenograft model of human breast cancer, curcumin in connection with paclitaxel (Taxol) prevented breast cancer metastasis to the lung to a greater degree than either curcumin or paclitaxel alone (Frank *et al.*, 2003).

Curcumin enlarges mucin secretion in rabbits and works as gastroprotectant against irritants (Lee *et al.*, 2003).

Ethanollic extract of turmeric composed of both curcuminoids and sesquiterpenoids was more strongly hypoglycemic than curcuminoids or sesquiterpenoids alone (Nishiyama *et al.*, 2005).

Ethanollic extract of turmeric had protective effect against gastric ulcers by blocking H₂ histamine receptors (H₂R) of male rats. Orally administered ethanollic extract prevented gastric acid and gastric juice secretion and ulcer formation comparable to the characteristics of ranitidine (Kim *et al.*, 2005).

An experiment was conducted to study Alzheimer's disease (AD) in animal models and result showed that curcumin could lower the amyloid pathology of the disease (Ringman *et al.*, 2005).

Curcumin showed multiple actions in brain based on many studies. It may be a future drug of therapy for the treatment of various neurological untidiness such as major depression, tardive dyskinesia and diabetic neuropathy (Kulkarni and Dhir, 2010).

As revealed from the review noted above curcumin has a wide range of medicinal effects on human body. The major functions of curcumin in human body are shown in the flow chart in Fig. 2.1 below.

Functions of curcumin in human body

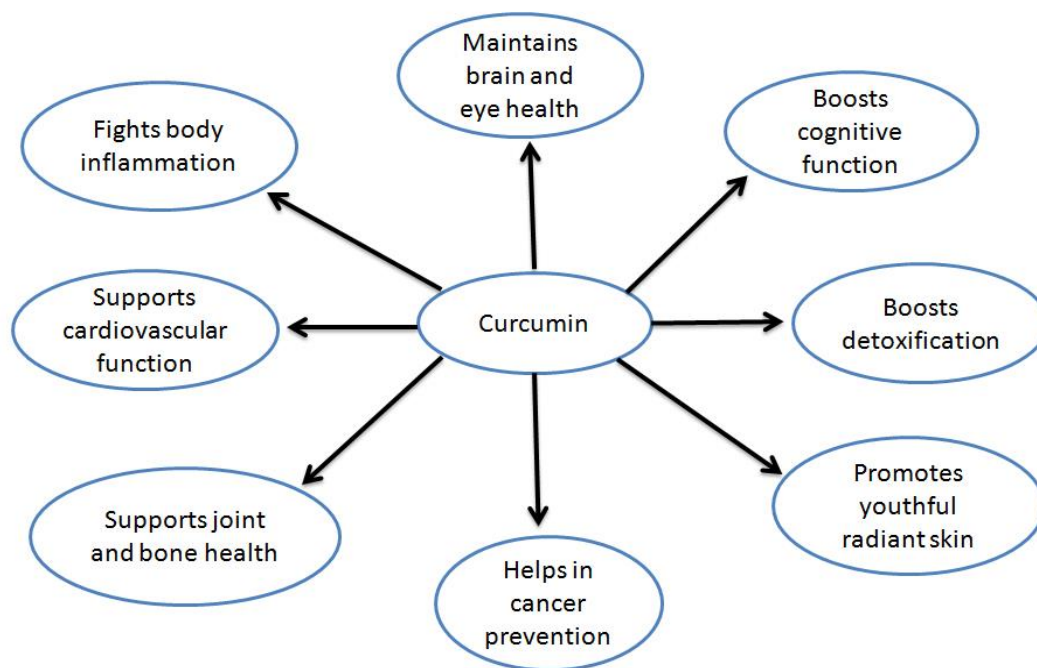


Fig. 2.1: Outline of major functions of curcumin in human body

Turmeric binds metals, particularly iron and copper, and can function as an iron chelator. It is remarkably nontoxic and shows limited bioavailability. The difference of curcumin content among the various genotypes of *C. longa* is caused by hybridization and introgression with other *Curcuma* species (Hayakawa *et al.*, 2011).

2.4 Standardization of protocol for micropropagation

Plant tissue culture or micropropagation is an assemblage of techniques used to grow plant cells, tissues or organs in a nutrient medium of specified composition maintained under sterile condition. It is mostly used to produce large number of clones or homogeneous copies of a plant having desirable characters. The technique relies on the fact that many plant cells have the property of totipotency.

The process of tissue culture has been defined differently by various workers as given below.

Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of true to the type progeny plants with the objectives of mass propagation, rapid clonal multiplication, conservation of important plant species and making the stock disease free (Philip and Gamborg, 2005).

In tissue culture large number of true to the type plants can be produced under controlled *in vitro* conditions in short time using plant material through explant selection, culture establishment, regeneration and acclimatization of the plantlets to *ex vivo* conditions (George *et al.*, 2008).

2.4.1 Micropropagation of *Curcuma longa* L.

A number of studies with different media composition have been carried out for shoot multiplication in *C. longa* as described below.

Rhizome buds of *C. longa* were inoculated on Murashige and Skoog (MS) medium with varying levels of BAP and kinetin. This experiment confirmed that 0.1 mg/L kinetin and 0.2 mg/L BAP was the best combination for shoot formation. The combination of 0.1 mg/L kinetin and 0.2 mg/L BAP was best resulting in formation of 7-8 healthy green shoots. The survival rate of plants transferred to the field was 80% (Nadgauda *et al.*, 1978).

Maximum shoot production in turmeric cv. Elite was observed in MS medium containing 10 pM BA and 1 pM NAA and solidified with agar at 0.4% and 0.6% and was found to be superior to liquid media for the same purpose. Effect of various carbohydrate sources was investigated on shoot multiplication *in vitro* of *Curcuma longa* and glucose was found to be more efficient for shoot multiplication. Further, carbohydrate sources like xylose, rhamnose, lactose and soluble starch were inhibitory for shoot multiplication. The positive effect of kinetin (10 pM) along with NAA (1 pM) was also observed in shoot multiplication of *C. longa* (Salvi *et al.*, 2002).

MS medium containing 5.0 mg/L BAP + 2.0 mg/L NAA and 3.0 mg/L BAP + 0.5 mg/L NAA gave nearly 5.65 shoots per explant of turmeric. The longest shoot length (9.64 cm) was found on the media containing 1.0 mg/L of BAP + 2.0 mg/L of NAA and 5.0 mg/L of BAP + 2.0 mg/L of NAA respectively (Ikeda and Tambe, 1989).

For shoot multiplication, MS medium supplemented with 1 mg/L BAP and 0.5 mg/L NAA showed the best result producing 8-10 shoots in 40 days after inoculation whereas MS medium containing 5.0 mg/L BAP + 2.0 mg/L NAA and 3.0 mg/L BAP + 0.5 mg/L NAA gave nearly 5.65 shoots per explant (Nirmal Babu *et al.*, 1993 and 1997).

In a study maximum number of shoots per bud was obtained during culture initiation on the medium containing 2 mg/L kinetin (7.7 shoots/explant) which was significantly higher than the number of shoots obtained on MS basal medium and MS + kinetin 8 mg/L (4.3 shoots/explant) (Sharma and Singh, 1997).

High frequency of shoot multiplication was observed in *C. longa* using thidiazuron. In this study, both undivided and divided terminal buds were used as explants in MS medium supplemented with TDZ (0.23-18.17 pM). After inoculation in TDZ (18.17 pM), explants were subcultured on MS basal medium. A number of 3.42 to 5.08 shoots from undivided buds and 3.47 to 4.36 shoots from divided buds were obtained. On subculturing after 4 weeks in MS basal, 13.25 ± 0.70 shoots regenerated from undivided and 11.3 ± 0.86 shoots from divided explant. A maximum number of 18.22 ± 0.62 shoots were obtained in MS basal after 12 weeks (Prathanturarug *et al.*, 2003).

A few works on successful root multiplication in *C. longa* using various media composition were undertaken as detailed below.

An experiment was carried out with PTS 28 and summer cultivar of turmeric. From that experiment, successful root formation was observed on half strength MS medium supplemented with IAA or IBA at 0.25–0.5 mg/L and 2% sucrose (Rout *et al.*, 1995).

The elongated shoots of turmeric produced 9.66 ± 1.20 roots on half strength MS medium supplemented with 2 mg/L of IBA and showed 86% survivability after hardening (Ghosh *et al.*, 2013).

Pathogen-free, uniform planting material of turmeric (*Curcuma longa* L.) was established through micropropagation method using emerging buds from the sprouting rhizomes as explants on MS medium containing BAP (1.0 mg/L) singly and BAP (1.0 mg/L) along with kinetin (0.25 mg/L). It was also reported that the rooting of the microshoots was observed on MS medium containing 1.0 mg/L NAA (Ali *et al.*, 2004).

Callus induction and regeneration in *Curcuma* species under different media composition was also observed as described below.

The immature inflorescence (5mm) of *C. longa* was cultured in MS medium with BA (5 or 10 mg/L) in combination with 2,4-D (0.2 mg/L) or NAA (0.1 mg/L)

and TDZ (1 or 2 mg/L) in combination with IAA (0.1 mg/L). After four weeks, shoot primordia emerged from the surface of the explant in all cases. Among the different hormone combinations, BA (5 mg/L) and 2,4-D (0.2 mg/L) showed highest frequency of regeneration (98%) but TDZ (1 or 2 mg/L) in combination with IAA (0.1 mg/L) showed very low regeneration frequency (10%). The number of shoots (8.9 shoots) per inflorescence of explant was highest in TDZ (2 mg/L) and IAA (0.1 mg/L) combination (Salvi *et. al.*, 2000).

A combination of NAA (1.0 mg/L) with kinetin (1.0 mg/L) or NAA (1.0 mg/L) with BAP (2.0 mg/L) was found optimum for rapid clonal propagation of turmeric. A concentration of 2.5-3.0 mg/L of 2,4-dichlorophenoxy-acetic acid (2,4-D) was found optimum for callus induction. Regeneration of plantlets from a callus was successfully conducted in MS medium supplemented with standard growth hormones for multiplication at $25 \pm 2^\circ\text{C}$ under a 16 h photoperiod. These plantlets were successfully transferred to the field. Plantlets (4 months old) were incubated in a medium containing different concentrations of sucrose supplemented with NAA (0.1 mg/L) and Kinetin (1.0 mg/L) at $27 \pm 2^\circ\text{C}$ under an 8 h photoperiod for induction of rhizomes. *In vitro* rhizome formation was observed in media containing 6 and 8% sucrose (Sunitibala *et. al.*, 2001).

An experiment was conducted on MS nutrient medium supplemented with various growth regulators to observe callus induction and regeneration of *Curcuma amada* and *Curcuma aromatica*. High rate of callus formation was found at concentration of 2 mg/L of 2,4-D (Prakash *et al.*, 2004).

Soft and friable callus was found to be formed from turmeric (*Curcuma longa* L.) buds grown on MS medium supplemented with 2 mg/L IAA or 0.5 mg/L 2,4-D and kept in dark. When exposed to light, IAA induced calli underwent differentiation to produce several plantlets. These plantlets could be differentiated and grown further before they were transplanted to polybags (Kuruvinashetty and Iyer, 1980).

Callus cultures were initiated from leaf bases of turmeric on MS basal medium supplemented with dicamba, piclorum (2 mg/L) or NAA (5 mg/L) in combination with BA (0.5 mg/L). On transfer of callus cultures to medium supplemented with BA (5 mg/L) in combination with triiodobenzoic acid (TIBA) or

2,4-D (0.1 mg/L), green shoot primordia were separated from the surface of the callus (Salvi *et al.*, 2001).

2.4.2 Micropropagation of *Curcuma caesia* Roxb.

Curcuma caesia Roxb., commonly known as black turmeric or Kali haldi, is a rhizomatous erect herb of about 0.5 to 1.0 m high. The broadly lanceolate leaves are glabrous and 30–60 cm long and up to 15 cm broad with a ferruginous purple band running along the middle of the lamina. Flowers are pale yellow, reddish at the outer sides and short in length. The inflorescence is 15-20 cm long dense spike with green bracts. The ovoid shaped rhizomes are thin and acute at tip and are used as propagation material. It is native to North-East and Central India and mostly found in Bengal, Arunachal Pradesh, Meghalaya and Mizorum (Das *et al.*, 2013).

A number of works on the micropropagation of this species have been carried out by different workers which are summarized below.

Shoot multiplication in *Curcuma caesia* was found to occur in MS medium in presence of different growth regulators in the following studies.

Sterilized rhizome buds of *Curcuma caesia* were cultured on MS medium supplemented with or without BAP (5 mg/L). The optimum length of rhizomes to produce microshoots at initial stage cultured on medium supplemented with 5 mg/L BAP was more than 2 cm. For microshoot propagation, explants were also cultured on medium supplemented with different concentrations and combinations of BAP and NAA. Explants cultured on MS basal medium supplemented with 3 mg/L BAP showed highest proliferation rate (95%) where average number of microshoots produced was 9.5 (Zuraida, 2013).

For shoot multiplication of *C. caesia* 3.0 mg/L BA supplemented with MS medium was found to be optimum (Balachandran *et al.*, 1990).

In another study, 4.0 mg/L BA + 1.5 mg/L NAA in MS medium was found to be the best growth regulator combination for shoot multiplication of *C. caesia* (Bharalee *et al.*, 2005).

The optimum shoot proliferation was found in case of black turmeric from MS medium containing 3.0 μ M BA + 0.5 μ M NAA and produced 10.38 shoots with 4.53 cm length after 8 weeks of culture (Shahinozzaman *et al.*, 2013).

In the study conducted with *C. caesia* in MS medium supplemented with 2.5 mg/L of BAP and 1.5 mg/L of NAA, 9.00 ± 0.57 number of shoots were observed to grow with an average length of 7.20 ± 1.01 cm (Ghosh *et al.*, 2013).

For root multiplication in *C. caesia* MS medium supplemented with a combination of 4.0 mg/L BAP and 2 mg/L IAA gave the highest number of roots. A survival rate of 80% was observed when healthy and complete plants with well-developed roots were hardened, acclimatized and planted in the field (Singh *et al.*, 2015).

In vitro microshoots of *Curcuma caesia* subcultured on full strength MS media supplemented with 3 mg/L BAP and 0.5 mg/L NAA performed better in terms of the number of shoots as well as root initiation. Rooted shoots transplanted in the glasshouse for hardening showed 95% survival rate (Zuraida, 2013).

NAA was found to be best auxin over IBA for *in vitro* rooting of *C. caesia* in half strength MS medium containing NAA and IBA alone at different concentrations (0.0, 1.0, 3.0, 3.5 4.0 and 4.5 μ M). Maximum $89.76 \pm 3.79\%$ shoots produced 9.73 ± 1.07 roots when cultured into medium containing 3.0 μ M IBA (Shahinozzaman *et al.*, 2013).

Callus induction and regeneration in *Curcuma caesia* were done successfully in a few studies as enumerated below.

Callus induction was found to occur in presence of 2,4-D at concentrations of 2.7-3.4 mg/L. Regeneration of plantlets from a callus was successful in MS medium supplemented with a combination of 2.5 mg/L BAP and 1.0 mg/L NAA for multiplication at 26°C under 24 h photoperiod (Singh *et al.*, 2015).

The highest mean number of callus (3.44 ± 0.98) was found to be formed in *Curcuma caesia* in presence of 2.5 mg/L of picloram. A number of 1.33 ± 0.46 callus was formed in presence of 2.0 and 4.0 mg/L of 2,4-D. As an effect of 2,4 D in association with BAP, the highest mean number of callus (1.99 ± 0.67) was formed in 1.0 mg/L 2,4 D with 0.5 mg/L BAP and 3.0 mg/L 2,4-D with 0.5 mg/L BAP (Md Zain, 2013).

The explants of *Curcuma caesia* were cultured on Woody Plant Medium (WPM) together with different concentrations of 2,4-Dichlorophenoxyacetic acid (2,4-D) and benzyl aminopurine (BAP) in presence of light. The growth medium

supplemented with 5 mg/L BAP and 2 mg/L 2,4-D promoted callus induction after 70 days of culture. Sub-culturing on the same medium enhanced the production of friable callus. Culture media containing higher concentrations of agar promoted the development of green somatic embryos from the callus. Respond of somatic embryogenesis was most successful with MS medium in 6.0 g/L agar supplemented with 5 mg/L BAP and 0.2 mg/L 2,4-D whereby the callus developed green somatic embryos with an efficiency of 53%. This medium also produced the largest number plantlets (Zuraida *et al.*, 2014).

Materials and Methods

The materials used, methods employed and statistical techniques adopted for scoring of the collected genotypes of turmeric based on statistical analysis of phenotypic and biochemical characters, estimation of some quality determining biochemical parameters and standardization of protocol for micropropagation of best of the selected cultivars have been described in this chapter.

3.1 Field Study

3.1.1 Location of the experimental site

The field experiments were conducted during the period from 2015-16 to 2017-18 at the Instructional Farm of the Faculty of Agriculture, Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal, India. The experimental farm is situated at the Terai agro-climatic zone of West Bengal between 26°19' 86" N latitude and 89°23.5" E longitude at an altitude of 43.0 m above mean sea level. The topography of the land is medium upland bearing good drainage and irrigation facilities.

3.1.2 Agro-climatic conditions of the experimental area

The agro-climatic condition of this zone is characterized by high rainfall and high relative humidity with moderate temperature. The minimum temperature varies from 9.48°C to 25.99°C and maximum temperature varied from 23.84°C to 34.16°C. The average rainfall of this zone is 3000 mm. The meteorological data observed during the period of study is presented below (Table 3.1).

Meteorological features during the period of experiment

Rainfall: The total amount of rainfall during the field experiment of turmeric was 2393.80 mm, 3155.95 mm and 3043.60 mm in the year 2015-16, 2016-17 and 2017-18 respectively. The highest rainfall occurred during the months of June to September.

Table 3.1: Meteorological data during the period of study

Month	Year*	Rainfall (mm)	Temperature (°C)		Relative humidity (%)	
			Maximum	Minimum	Maximum	Minimum
May	Y ₁	377.80	31.77	22.29	80.00	76.65
	Y ₂	364.80	31.02	21.72	83.61	76.42
	Y ₃	391.60	31.78	22.34	90.45	71.90
June	Y ₁	622.00	31.20	23.90	91.40	82.87
	Y ₂	886.20	32.77	23.61	88.97	79.43
	Y ₃	502.50	32.80	24.66	92.00	76.00
July	Y ₁	296.90	33.42	25.12	84.49	74.97
	Y ₂	846.20	31.48	25.35	93.26	85.65
	Y ₃	413.10	32.12	25.49	91.00	78.00
August	Y ₁	797.30	31.77	25.15	88.89	81.10
	Y ₂	412.85	34.16	25.99	85.90	78.42
	Y ₃	979.50	32.22	25.63	96.97	80.58
September	Y ₁	299.80	32.88	24.92	78.53	71.63
	Y ₂	498.20	32.16	24.79	91.07	86.10
	Y ₃	532.40	32.88	25.10	95.97	76.47
October	Y ₁	0.00	32.59	22.33	72.62	67.74
	Y ₂	147.70	32.84	21.81	82.00	74.32
	Y ₃	224.50	30.84	22.31	96.35	74.39
November	Y ₁	0.00	29.28	22.66	81.67	74.87
	Y ₂	0.00	32.77	21.58	81.58	73.65
	Y ₃	0.00	29.51	15.69	94.83	55.40
December	Y ₁	0.00	25.11	18.16	83.26	79.32
	Y ₂	0.00	32.68	21.36	81.32	72.97
	Y ₃	0.00	26.94	12.78	97.26	55.42
January	Y ₁	0.00	23.84	9.48	91.19	81.97
	Y ₂	0.00	26.28	9.66	97.00	46.84
	Y ₃	0.00	21.84	9.25	94.94	60.42

* Y₁, Y₂ and Y₃ represent 2015-16, 2016-17 and 2017-18 respectively

Temperature: Temperature was higher at the beginning and then gradually decreased during the experimental period. Maximum temperature varied from 23.84°C to 34.16°C while minimum temperature varied from 9.48°C to 25.99°C during February.

Relative humidity: The maximum relative humidity varies from 72.62% to 97.26% and minimum relative humidity varies from 46.84% to 86.10% during the three years.

3.1.3 Experimental details

The details of the experiments carried out were as follows:

- Years of study : 2015-16, 2016-17 and 2017-18
- Design of the experiment : Randomized Block Design (RBD)
- Replications : 3 (three)
- Spacing : 30 cm x 20 cm
- Recommended dose of fertilizers : N, P₂O₅ and K₂O @ 120:60:60 kg/ha
- Time of sowing : First week of May

3.1.4 Collection of genotypes

A total of sixty turmeric genotypes were collected from the stock maintained at the All India Coordinated Research Project (AICRP) on Spices, Uttar Banga Krishi Viswavidyalaya, Coochbehar Centre. Most of these genotypes were collected from different locations of Terai and Dooars plains of northern part of West Bengal along with a few from southern part of West Bengal and from Koraput district of Odisha.

The curcumin contents of these genotypes were estimated by UV-VIS spectroscopy method as detailed in Section 3.2.4.2. Based on the curcumin content (Table 4.3 of Chapter 4: Results and Discussion) the genotypes were then divided into three groups namely Group I having low curcumin content (0.10 - 1.00%), Group II having medium curcumin content (1.01 – 2.50%) and Group III having high curcumin content (2.51- 5.50%). Best 15 (fifteen) from each group were taken, making a total of 45 (forty five) genotypes for all subsequent studies. The list of the selected genotypes along with their sites of collection is given in Table 3.2.

Table 3.2: Sites of collection of selected genotypes used in the present study

Sl. No.	Genotype	Site of Collection		
		Locality	District	State
1.	TCP 2*	Bararangras Village	Coochbehar	West Bengal
2.	TCP 7	Baxirhat Sabji Bazar	Coochbehar	West Bengal
3.	TCP 11	Neelpara Forest	Jalpaiguri	West Bengal
4.	TCP 12	Neelpara Forest	Jalpaiguri	West Bengal
5	TCP 34	RRS, UBKV	Darjeeling	West Bengal
6	TCP 40	RRS, UBKV	Darjeeling	West Bengal
7	TCP 50	RRS, UBKV	Darjeeling	West Bengal

Contd..... next page

Sl. No.	Genotype	Site of Collection		
		Locality	District	State
8	TCP 54	RRS, UBKV	Darjeeling	West Bengal
9	TCP 57	OUAT	Koraput	Orissa
11	TCP 58	Dumdim	Jalpaiguri	West Bengal
12	TCP 63	Tea Estate Basti	Alipurduar	West Bengal
13	TCP 64	Tea Estate Basti	Alipurduar	West Bengal
14	TCP 67	Baxirhat	Coochbehar	West Bengal
15	TCP 84	Rajabhatkhawa Forest	Alipurduar	West Bengal
16	TCP 87	Rajabhatkhawa Forest	Alipurduar	West Bengal
17	TCP 93	Rajabhatkhawa Forest	Alipurduar	West Bengal
18	TCP 94	Damdima Village	Jalpaiguri	West Bengal
19	TCP 96	Damdima Village	Jalpaiguri	West Bengal
20	TCP 100	Damdima Village	Jalpaiguri	West Bengal
21	TCP 114	Gairkata	Jalpaiguri	West Bengal
22	TCP 137	Gorumara Basti	Jalpaiguri	West Bengal
22	TCP 138	Gorumara Basti	Jalpaiguri	West Bengal
23	TCP 139	Keunta	Burdwan	West Bengal
24	TCP 152	Burdwan Town Market	Burdwan	West Bengal
25	TCP 154	Naraharipur	Midnapur	West Bengal
26	TCP 173	Salas Chiravata	South Dinajpur	West Bengal
27	TCP 175	Tunivata	North Dinajpur	West Bengal
28	TCP 178	Ghardhappa, Gandabari	North Dinajpur	West Bengal
29	TCP 181	Nijamatpur, Panjipara	Paschim Burdwan	West Bengal
30	TCP 194	UBKV, Pundibari	Coochbehar	West Bengal
31	TCP 195	Totopara	Alipurduar	West Bengal
32	TCP 196	Howrah South	Jalpaiguri	West Bengal
33	TCP 198	Kharia	Jalpaiguri	West Bengal
34	TCP 200	Jalpesh	Jalpaiguri	West Bengal
35	TCP 202	Changrabanda	Coochbehar	West Bengal
36	TCP 221	Mukurar, Jamaladaha	Coochbehar	West Bengal
37	TCP 222	Meghnasa Nagar	Jalpaiguri	West Bengal
38	TCP 224	Meghnasa Nagar	Jalpaiguri	West Bengal
39	TCP 225	Uttar Madarihat	Jalpaiguri	West Bengal
40	TCP 227	Hamtapara, TG	Jalpaiguri	West Bengal
41	TCP 240	Fatepur	North Dinajpur	West Bengal
42	TCP 242	Fatepur	North Dinajpur	West Bengal
43	TCP 244	Neamatpur	Paschim Burdwan	West Bengal
44	TCP 247	Neamatpur	Paschim Burdwan	West Bengal
45	TCP 276	Pundibari	Coochbehar	West Bengal

* TCP stands for Turmeric Collection from Pundibari

3.1.5 Crop management practices

The following crop management practices were employed for cultivation of the collected genotypes of turmeric.

3.1.5.1 Land preparation

Experimental field was ploughed to a fine tilth to break the clods and pulverized through tractor ploughing. Weeds and stubbles of the previous crop were removed and cleaned followed by breaking the clods. Proper leveling was done after each ploughing.

Total experimental area was divided into unit plots of 3 m x 1 m size having three replications. Channels of 40 cm width demarcated each replication and each plot was separated by 25 cm wide furrows. Each bed was prepared with 30 cm x 20 cm spacing between the rows for proper irrigation.

3.1.5.2 Sowing of the genotypes

Mother rhizomes of all the genotypes with well developed buds were used for planting in the field. Sowing was done during the first week of May.

3.1.5.3 Fertilizer application

In the experimental plots, a fertilizer dose of N: P₂O₅: K₂O @ 120:60:60 kg/ha were applied. The nitrogenous fertilizer was applied in three equal split doses at 45, 75 and 110 days after planting of turmeric rhizomes. Total amount of phosphorus and potassium fertilizers were applied as basal dose. Urea, SSP and MOP as source of NPK were applied on the basis of plot size. Farm Yard Manure (FYM) was applied @ 15 t/ha in the plots during land preparation.

3.1.5.4 Crop management

Three weeding followed by hoeing were done to make the field of weeds free. The plant protection measures were taken, as required, to control the attack of insect pests and weeds in the field. Irrigation was applied as per requirement.

3.1.5.5 Harvesting

Harvesting of turmeric crop was done during the first week of February after complete maturity of the crop as indicated by the drying up of leaves and falling down of plants. The rhizomes were dug out after cleaning the dry shoots from the field. Then adhering dirt and sticking roots were removed carefully and kept in cool and dry place.



Plate 3.1: Field of turmeric genotypes

3.1.6 Phenotypic growth characters study

A total of seven phenotypic characters were studied in the present experiment. Some randomly chosen plants in each plot were labeled and used for recording the data of the phenotypic characters as mentioned below.

3.1.6.1 Plant height

Plant height was measured in five randomly selected plants at 120 days after planting (DAP) from the ground level to the tip of the leaf of the main shoot with the help of meter scale and their average was expressed in centimeter.

3.1.6.2 Number of tillers per plant

The total number of tillers present in five randomly selected plants was counted at 120 DAP and their average was expressed as total number of tillers per plant.

3.1.6.3 Number of leaves per plant

The number of leaves in five randomly selected plants was counted at 120 DAP and their average was expressed as number of leaves per plant.

3.1.6.4 Length of leaf

The length of leaf in five randomly selected leaves was measured from the leaf base to the tip of leaf along the midrib at 120 DAP and their average was calculated in centimeter.

3.1.6.5 Leaf breadth

Leaf breadth was measured by the length at the widest point of the leaf lamina from the top in the tallest tiller of the plant at 120 DAP in five randomly selected plants and their average was expressed in centimeter.

3.1.6.6 Rhizome weight

The weight of fresh and whole rhizome of five randomly selected plants was measured in gram after the maturity of crop and their average was expressed as rhizome weight per plant.

3.1.6.7 Yield

The yields of mother rhizome for all the replicate plots were measured. Their average was taken and expressed in tonnes per hectare.

3.2 Laboratory Study

The soil and plant sample analyses associated with the present study were carried out at the laboratory of Department of Biochemistry, Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, West Bengal.

3.2.1 Chemicals and reagents

All the chemicals and reagents (AR grade) used in the experiments enumerated below were of Sigma Aldrich Inc, USA; Merck Specialities Pvt. Ltd, Mumbai, India and SRL Pvt. Ltd, Mumbai, India. Double distilled as well as Milli-Q water were used in the laboratory works as per requirement.

3.2.2 Instruments

The following instruments were used in the present study:

- 1) Mixer grinder – model no. HL1618, make – Philips, India
- 2) Cold centrifuge – model no. 2-16KL, make – Sigma, Germany
- 3) UV–VIS spectrophotometer – model no. UV1800, make – Shimadzu, Japan
- 4) pH meter – model no. pH 700, make – Eutech, Singapore
- 5) Oil extractor (Socs Plus) – model no. SCS 04R, make – Pelican, India
- 6) Colorimeter – model no. Color Quest XE S/N-CQX 3031, make – Hunter Lab, USA
- 7) Atomic Absorption Spectrophotometer - AAnalyst 200, Perkin Elmer, Singapore
- 8) Weighing balance – model no. ME203E, make – Mettler Toledo, Switzerland
- 9) Ice bath – model no. 2006030303952, make – Blue Star, Italy

All the measuring glassware was class A type made of Borosil Glass Works Ltd, Mumbai, India.

3.2.3 Physico-chemical analysis of soil

The composite soil samples were collected from 0–15 cm depth of the experimental field with the help of a soil auger before the application of fertilizers. The soil samples were then dried thoroughly under shade, pulverized and sieved through a 0.2 mm sieve for physico–chemical analyses.

Soil pH was estimated by potentiometric method using pH meter (Baruah and Barthakur, 1997). Organic carbon was assayed by rapid titration method (Walkley and Black, 1934). The modified micro–Kjeldahl method was applied to estimate the

available nitrogen in the soil (Jackson, 1967). Available phosphorus was measured by Bray's No. I method (Jackson, 1967) and available potassium content was done by flame photometer method (Jackson, 1967).

3.2.4 Biochemical analyses

The biochemical analyses carried out in the present study included quantitative estimation of curcumin, protein, total phenol, crude fat and iron; assay of activity of enzymes namely catalase, peroxidase and superoxide dismutase; assay of antioxidant activity by ABTS, DPPH, TAC and CUPRAC methods; rhizome colour and measurement of pH changes of rhizome powder by the methods as described below.

3.2.4.1 Sample preparation

Turmeric rhizomes of all the genotypes under study were collected from field, roots were removed and washed with water. Then the rhizomes were taken in separate beakers and kept under shade for drying. Afterwards they were ground with a mixer grinder in sufficient amount, taken in air tight plastic containers with proper labeling and kept in desiccator.

3.2.4.2 Curcumin estimation

The curcumin content in turmeric samples was estimated by UV-VIS spectroscopy as per method described by Jansirani *et al.* (2014) with some modifications. Ten mg of turmeric powder was dissolved in methanol taken in a 10 ml volumetric flask to make a stock solution of concentration of 1000 ppm. It was then diluted to 200 ppm with methanol in a 5 ml volumetric flask.

This solution was used for UV-VIS spectroscopic estimation by taking in a 3 ml quartz cuvette and measuring its absorbance at 425 nm in a UV-VIS spectrophotometer. The amount of curcumin present in the samples was calculated from the standard curve prepared by plotting concentration of standard curcumin on the x-axis versus absorbance on the y-axis. Amount of curcumin present in dry turmeric powder was expressed as percent value.

3.2.4.3 Protein estimation

The protein content from the turmeric powder was estimated as per the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as standard. The standard stock solution was prepared by dissolving 50 mg BSA in 50 ml distilled

water and the working standard was made by diluting the stock solution to 1:5 dilutions with distilled water.

For protein extraction, 500 mg of turmeric powder was mixed well with 10 ml sodium phosphate buffer (0.1 M, pH 7.0) taken in a pre-chilled mortar and pestle. It was then mixed thoroughly and centrifuged at 10,000 rpm for 20 minutes and the supernatant was collected for protein assay.

The following reagents were prepared.

Reagent A : 1 g Na_2CO_3 in 50 ml of 0.1 (N) NaOH

Reagent B : 0.25 g $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ in 50 ml of 1% potassium sodium tartrate

Reagent C : 50 ml reagent A + 1 ml reagent B

Reagent D : 1 (N) Folin–Ciocalteu reagent

Protein extract (0.1 ml) was taken with a micropipette in a test tube and its volume was made up to 1 ml. Five ml of reagent C was added to it and kept for 10 minutes. To it 0.5 ml of reagent D was added, stirred well and incubated at room temperature in dark for 30 minutes. Blue colour was developed. The absorbance was measured at 660 nm in a UV–VIS spectrophotometer against a reagent blank without having protein extract. Amount of protein present was calculated with the help of a standard curve prepared by plotting concentration of standard BSA on the x-axis versus absorbance on the Y-axis. Amount of protein present was expressed as mg/g dry weight of rhizome powder.

3.2.4.4 Total phenol estimation

Total phenol content in turmeric rhizome powder was quantified by the Folin–Ciocalteu method as described by Sadasivam and Manickam (1996). Briefly, 500 mg turmeric powder was ground well in a mortar and pestle after mixing with 5 ml of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes and the supernatant was saved. The residue was then re-extracted with 5 ml of 80% ethanol following the same method and the supernatants were pooled and evaporated to dryness on hot water bath. The residue was dissolved in 5 ml of distilled water.

Then 0.4 and 0.5 ml of the aliquots was pipette out into separate test tubes. The volume in each tube was made up to 3 ml with distilled water and 0.5 ml of 1 N

Folin–Ciocalteu reagent was added. The mixture was allowed to react for 3 min. Then 2 ml of 20% Na_2CO_3 solution was added and mixed well. The tubes were placed in boiling water bath for exactly one minute and cooled. The absorbance was measured at 650 nm immediately in a UV-VIS spectrophotometer. The total phenol content was estimated in gallic acid equivalent using gallic acid standard curve and was expressed as mg/g dry rhizome powder.

3.2.4.5 Crude fat estimation

The estimation of crude fat was carried out with automatic solvent and fat extraction system using cellulose extraction thimbles (25 mm x 80 mm). Initially the weight of empty Soxhlet flask was recorded. One gram sample was weighed and poured into the thimble. Petroleum ether (b.p. 60-80°C) was poured into the thimble keeping the sample dipped into the solvent. The instrument was run and after completion of the extraction the solvent got evaporated totally. The flask of Soxhlet was then taken from the instrument and placed in the hot air oven for 10 minutes. It was then cooled and weighed again. The difference in weight of the latter with the blank flask gave the measure of crude fat content which was expressed in ml/100 g sample.

3.2.4.6 Iron estimation

The iron content in turmeric rhizomes was estimated by the method of Tandon (1998). Briefly, 1 g turmeric powder was placed in 100 ml conical flask. To this, 10 ml of acid mixture (9:4 of $\text{HNO}_3\text{:HClO}_4$) was added and the content of the flask was mixed by swirling. The conical flask was capped tightly and was kept for overnight. The flask was placed on sand bath at 180°C temperature until the production of red nitrogen dioxide (NO_2) fumes ceased. The contents were further evaporated until the volume was reduced to about 3 to 5 ml but not to dryness. The completion of digestion was confirmed when the liquid became colorless. After cooling the flask, 20 ml of deionized or glass distilled water was added and poured in 100 ml volumetric flask. Volume was made up with deionized water and the solution was filtered through Whatman No. 1 filter paper. Aliquots of this solution were used for the determination of iron by Atomic Absorption Spectrophotometer. The condition of the instrument was checked using CuSO_4 solution and then was standardized with FeSO_4 solution before the samples were measured. The iron content was expressed as mg/100 g of sample.

3.2.4.7 Assay of enzyme catalase

The estimation of enzyme catalase was done by the method of Sadasivam and Manickam (1996) with some modifications. Briefly, 0.1 g of fresh rhizome was crushed uniformly in a pre-cooled mortar and pestle after addition of 2 ml phosphate buffer (M/15; pH = 7.0). The homogenate was centrifuged at 10,000 rpm at 4°C for 30 minutes in a cold centrifuge and the supernatant was used as enzyme source.

In a cuvette 0.2 ml of enzyme extract, 2.3 ml of phosphate buffer (M/15) and 0.5 ml of H₂O₂ (0.2 M) were taken. The change in absorbance was recorded at 240 nm at 30 seconds interval for 5 minutes in a UV-VIS spectrophotometer. The enzyme activity was expressed as “ $\Delta A_{240}/\text{min/g}$ fresh weight”, where ΔA_{240} represents the change in absorbance at 240 nm.

3.2.4.8 Assay of enzyme peroxidase

The enzyme peroxidase was assayed by the method of Sadasivam and Manickam (1996) with some modifications. 0.1 g of fresh rhizome was taken in a pre-cooled mortar and pestle. Then 2 ml phosphate buffer (0.1 M; pH = 6.5) was added and crushed uniformly. The homogenate was centrifuged at 10,000 rpm at 4°C for 30 minutes in a cold centrifuge. The supernatant was used as enzyme source.

In a cuvette 0.2 ml of enzyme extract, 2.5 ml of phosphate buffer (0.1 M; pH = 6.5), 0.1 ml of o-dianisidine (1 mg/ml in methanol) solution and 0.2 ml of H₂O₂ (0.2 M) were taken. The change in absorbance was measured at 430 nm at 30 seconds interval for 3 minutes in a UV-VIS spectrophotometer. The enzyme activity was expressed as “ $\Delta A_{430}/\text{min/g}$ fresh weight”, where ΔA_{430} represents the change in absorbance at 430 nm.

3.2.4.9 Assay of superoxide dismutase

The assay of the enzyme superoxide dismutase was done by the method of Sadasivam and Manickam (1996) with some modifications. Briefly, 0.1 g of fresh rhizome was taken in a pre-cooled mortar and pestle and 2 ml of phosphate buffer (100 mM; pH = 7.8) was added to it and crushed uniformly. The homogenate was centrifuged at 10,000 rpm at 4°C for 30 minutes in a cold centrifuge and the supernatant was used as enzyme source.

An assay mixture of volume 3 ml was prepared by mixing 2.0-2.4 ml of phosphate buffer (100 mM; pH = 7.8), 0.1-0.5 ml of enzyme extract, 0.1 ml of

riboflavin (5 mM), 0.1 ml of methionine (100 mM), 0.2 ml of EDTA (10 mM) and 0.2 ml of Nitroblue tetrazolium (NBT, 1.5 mM) salt solutions. Then a blank was set without enzyme and NBT to calibrate the spectrophotometer. Another control was set having NBT but no enzyme as reference control. All the tubes were exposed to 4 x 100 W bulbs for 15 min. The absorbance was recorded at 560 nm immediately after that in a UV-VIS spectrophotometer. The 50% inhibition of the reaction between riboflavin and NBT in presence of methionine was taken as 1 unit of SOD activity. The enzyme activity was expressed as units/mg of protein.

3.2.4.10 Assay of antioxidant activity

Preparation of rhizome extract

Dried rhizome powder of 1 g each was extracted with methanol by cold percolation method for 72 hours with occasional shaking. Each extract was filtered through Whatman no. 42 filter paper. The filtrate was concentrated by evaporating excess methanol at 35°C and a semi-solid residue was obtained. The residue was again dissolved in methanol to prepare stock solution and from stock solution different concentrations such as 10, 25, 50, 75, 100, 125, 150, 175 and 200 ppm were prepared.

3.2.4.10.a ABTS radical scavenging activity

The ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity was determined by the method of Thaipong *et al.* (2006). Two stock solutions of 7.4 mM ABTS and 2.6 mM potassium persulfate were prepared separately. Then working solution was prepared by properly mixing these two stock solutions in equal quantities and allowing them to react for 12 hours in the dark at room temperature. It was then diluted by mixing 1ml of working solution with 60 ml methanol to get an absorbance of 1.1 ± 0.02 units at 734 nm in UV-VIS spectrophotometer. A volume of 0.5 ml of the solvent extract was allowed to react with 2.5 ml of ABTS solution for 2 h in dark. The absorbance was measured at 734 nm using UV-VIS spectrophotometer and result was expressed in percent of scavenging activity. The IC_{50} value was calculated from the % inhibition vs. concentration of different sample extracts by comparing the absorbance values of control (A_{control}) and sample extract (A_{sample}). Radical scavenging activity was measured by the following equation:

$$\text{ABTS radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}})] / A_{\text{control}} \times 100$$

Where, A_{control} is the absorbance of ABTS radical + methanol; and

A_{sample} is the absorbance of ABTS radical + sample extract.

3.2.4.10.b DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was assayed by the method described by Thaipong *et al.* (2006). A stock solution of DPPH was made by dissolving 14 mg in 50 ml methanol and working solution was prepared by diluting 10 ml stock solution with 45 ml methanol to get an absorbance of 1.1 ± 0.02 units at 517 nm in UV-VIS spectrophotometer. Rhizome extract of 0.5 ml volume was then allowed for reacting with 2.5 ml of DPPH solution for half an hour in the dark. Thereafter the absorbance was recorded at 517 nm using UV-VIS spectrophotometer and results were expressed in percent of scavenging activity. The IC_{50} value was calculated from the % inhibition vs. concentration of different sample extracts by comparing the absorbance values of control (A_{control}) and sample extract (A_{sample}). Radical scavenging activity was measured by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}})] / A_{\text{control}} \times 100$$

Where, A_{control} is the absorbance of DPPH radical + methanol; and

A_{sample} is the absorbance of DPPH radical + sample extract.

3.2.4.10.c Total antioxidant capacity

Total antioxidant capacity (TAC) of the extracts was evaluated by phosphomolybdenum method described by Prieto *et al.* (1999). A volume of 0.3 ml rhizome extract was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) taken in separate test tubes. The test tubes were incubated at 95°C for 90 min and subsequently were cooled at room temperature. The absorbance of the test solution was measured at 695 nm using a UV-VIS spectrophotometer against blank. Total antioxidant activity is expressed as the number of miligram equivalent of ascorbic acid per gram sample. The standard curve was prepared by mixing ascorbic acid (10, 25, 50, 75, 100, 125, 150, 175 and 200 µg/ml) in methanol, with above reagent solution.

3.2.4.10.d CUPRAC antioxidant assay

The CUPRAC (Cupric Ion Reducing Antioxidant Capacity) antioxidant assay of the turmeric rhizomes was done by the method of Apak *et al.* (2004). One gram dried rhizome powder was crushed with 20 ml acetone-water mixture (7:3 v/v). The

content was then centrifuged at 3000 rpm for 10 min and the supernatant was collected and stored at 4°C.

One ml of each of ammonium acetate buffer (pH 7.0), copper (II) chloride (0.01 M) and neocuproine (7.5×10^{-3} M, prepared in 96% ethanol) were mixed in a test tube. To this, 100 μ l of sample extract was added and final volume was made up to 4.1 ml with distilled water and kept for 30 minute. The absorbance was recorded at 450 nm against a reagent blank. The CUPRAC assay is expressed as Trolox equivalent (mM TE/g) and is calculated by the formula:

$$\text{mM TE/g} = (A_f / \epsilon \text{TR}) \cdot (V_f / V_s) \cdot r \cdot (V_{\text{initial}} / m)$$

Where A_f = absorbance

$$\epsilon \text{TR} = 1.67 \times 10^4 \text{ Lmol}^{-1}\text{cm}^{-1}$$

V_f = final volume

V_s = volume of aliquot

r = dilution factor

V_{initial} = initial volume

m = weight of sample

3.2.4.11 Rhizome colour measurements

The color of rhizomes was measured by a portable colorimeter as per the method suggested by Schmitzer *et al.* (2010). The instrument was standardized through standard Color Quest XE CQX3031 before use. Optical cell was used for this purpose. The sample was filled up in the optical cell (50 mm) of the instrument and covered with black mount cap. After placing it in the instrument data was recorded.

The three coordinates of CIELAB system of color representation are: the L^* value corresponds to a dark-bright scale and represents the relative lightness of colors with a range from 0 to 100 (0 = black, 100 = white). Color parameters are: a^* (negative for green and positive for red) and b^* (negative for blue and positive for yellow); a^* and b^* extend from -60 to 60 . The hue angle (h°) is expressed in degrees from 0 to 360. In this system, 0° = red, 90° = yellow, 180° = green and 270° = blue. The angle of hue was calculated from L^* , a^* and b^* values by using the following formula:

$$\text{Hue angle} = \tan^{-1} (b^*/a^*)$$

3.2.4.12 pH measurement

The pH changes of dried rhizome powder were determined by mixing 0.5 g of dry rhizome powder with 5 ml of Milli-Q grade water. After 2 hour with occasional stirring, pH of the solution was measured with pH meter (Schmitzer *et al.*, 2010).

Curcumin powder was dissolved in ethanol and finally adjusted the concentration to 10 ppm and the pH of the curcumin solution was then adjusted to a range distributed over acidic to basic *i.e.* 4.47, 6.1, 7.15, 8.71 and 10.8. Curcumin solution with different pH was scanned in a UV-VIS double beam spectrophotometer in a range of 200-700 nm.

Similarly a 10 ppm FeCl₃ solution was prepared and scanned as above by maintaining similar experimental conditions. Thereafter, 5 ml of curcumin solution with 10 ppm concentration of different pH (4.47, 6.1, 7.15, 8.71 and 10.8) and 5 ml of 10 ppm FeCl₃ solution were allowed to react at room temperature in different test tubes, plugged with non-absorbent cotton and were incubated for a period of 30 min in complete darkness. After the incubation resulting solutions were scanned for a range of wave length varying from 200 nm to 700 nm with the help of double beam UV-VIS spectrophotometer.

3.2.5 Micropropagation of TCP 2 genotype of *Curcuma longa* L. and *Curcuma caesia* Roxb.

From the results of the analysis of the biochemical parameters as per methods described in the previous section (Section 3.2.4) it has been established that the genotype TCP 2, is biochemically superior one among the selected 45 genotypes. Furthermore, this genotype has the following noteworthy features:

1. It is the first released turmeric variety from UBKV.
2. It is one high yielding and disease resistant (tolerant to rhizome rot and leaf blotch, www.spices/res/in) turmeric cultivar that has suitably been adapted for cultivation in terai region of West Bengal and has also been popular among the turmeric growers.
3. The rhizomes of this cultivar are robust and orange yellow in color and so the cultivar has very good economic potential.

So this genotype is considered as one promising genotype for growing in the northern region of West Bengal. But lack of availability of disease-free, homogeneous and true to the type quality planting material of this genotype imposes hurdles for its widespread cultivation. This genotype has, therefore, been selected for standardization of protocol for micropropagation to fulfill one of the objectives of the present research programme.

Black turmeric (*Curcuma caesia* Roxb.), an important medicinal plant belonging to *Zingiberaceae* family, is currently under threat of extinction since its natural habitat is being destroyed through various human activities. It was, therefore, thought worthwhile to include the standardization of protocol for micropropagation of this species also in the present programme.

The details of methodology of micropropagation adopted for the above two have been done as per method described by Shahinozzaman *et al.* (2013) with some modifications as narrated in the following sections.

3.2.5.1 Explants for tissue culture

Rhizomes of Suranjana (TCP 2) genotype of *Curcuma longa* L. and *Curcuma caesia* Roxb. were collected from AICRP on Spices at Pundibari under Uttar Banga Krishi Viswavidyalaya.

3.2.5.2 Sterilization of glassware and materials

All the glassware and other materials were cleaned thoroughly with Extran (Merck). These were then dried in hot air oven at 60°C. The petri plates, jam bottles, distilled water, forceps, scalpels, needles, scissors and spatula used were sterilized in an autoclave at 120°C for 20 minutes and flame sterilization was also done at the time of inoculation.

3.2.5.3 Growth Regulators

The growth regulators like 6-benzylaminopurine (BAP), Kinetin (Kn), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), α -naphthaleneacetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2,4-D) were used in different concentrations.

3.2.5.4 Carbon source

Sucrose was used as the source of carbon at the rate of 30 g/L in the medium.

3.2.5.5 Gelling agent

Agar was used at the concentration of 8 g/L for solidifying the culture medium.

3.2.5.6 Distilled water

Single distilled water was used for preparation of stock solutions, growth regulators, nutrient media etc.

3.2.5.7 pH of the media

The final pH of the media was adjusted to 5.8 using 1 N NaOH.

3.2.5.8 Preparation of nutrient media

The Murashige and Skoog (1962) media was used in the present study. The appropriate quantities of various stock solutions including growth regulators, vitamins were added. Required quantity of sucrose was added to the stock solutions. Required quantity of agar was weighed and dissolved in distilled water by uniform heating and constant stirring to avoid formation of clumps. The final volume of the medium was made up with distilled water. After mixing well, the pH of the medium was adjusted to 5.8 using 1 N NaOH. Composition of the stock solutions for MS medium has been given in Table 3.3.

3.2.5.9 Sterilization of Media

The jam bottles containing the tissue culture media were sterilized at 120°C for 15 minutes in an autoclave. After autoclaving they were kept at room temperature.

3.2.5.10 Culture room conditions

The cultures were maintained on racks in tissue culture room under a photoperiodic regime of 16 hours light and 8 hours dark period. The culture room was maintained with a temperature of 25±2°C and 65-70% relative humidity.

Table 3.3: Composition of the stock solutions for MS medium

Component	Stock solution (g/L)	Final concentration (mg/L)
Macronutrients stock		
KNO ₃ (Potassium nitrate)	95.00	1900.00
NH ₄ NO ₃ (Ammonium nitrate)	82.50	1650.00
MgSO ₄ ·7H ₂ O (Magnesium sulfate heptahydrate)	37.00	370.00
KH ₂ PO ₄ (Monopotassium phosphate)	17.00	170.00
CaCl ₂ ·2H ₂ O (Calcium chloride dihydrate)	44.00	440.00
Micronutrients stock		
H ₃ BO ₃ (Boric acid)	0.62	6.2
MnSO ₄ ·4H ₂ O (Manganese sulfate tetrahydrate)	2.23	22.30
ZnSO ₄ ·7H ₂ O (Zinc sulfate heptahydrate)	0.86	8.6
NaMoO ₄ ·2H ₂ O (Molybdic acid sodium salt dehydrate)	0.025	0.250
CuSO ₄ ·5H ₂ O (Copper sulfate pentahydrate)	0.0025	0.025
CoCl ₂ ·6H ₂ O (Cobaltous chloride hexahydrate)	0.0025	0.025
KI (Potassium iodide)	0.083	0.83
Iron stock		
FeSO ₄ ·7H ₂ O (Ferrous sulfate heptahydrate)	2.785	27.85
Na ₂ EDTA (Disodium ethylenediamine tetraacetate dihydrate)	3.725	37.25

3.2.5.11 Transfer area and aseptic manipulation

All the procedures like surface sterilization, preparation, inoculation of explants and sub-culturing were carried out in a laminar air flow chamber. Before using the laminar air flow chamber, the working surface was cleaned and swabbed with 70% ethanol. Later the UV lamp was switched on for 30-45 minutes followed by the airflow for 10 minutes before use. During the process of transfer, the dissecting instruments were cleaned with 70% ethanol followed by a dip in the glass bead sterilizer. The hands were also swabbed with 70% ethanol before carrying out the work to ensure the aseptic condition. After completing the inoculation and transfer process, the laminar air flow bench was cleaned and swabbed with 70% ethanol.

3.2.5.12 Sterilization of explants

Each rhizome was sliced into small pieces (3 to 4 cm) with buds on them and washed with tap water. The rhizomes were briefly treated with few drops of Tween 20 for one minute and cleaned under running tap water for half an hour. The explants were thoroughly treated with fungicide Bavistin (0.5%) for one hour followed by bactericide Plantomycin (0.05%) for 15-20 minutes. They were then rinsed several times with sterile distilled water. Finally, inside the laminar air-flow cabinet, surface disinfection was done by 70% ethanol and buds were treated with 0.1% mercuric chloride solution for 5 minutes. Thereafter the buds were rinsed with autoclaved distilled water.

3.2.5.13 Inoculation of explants

The inoculation of explants into jam bottles containing full strength MS media was carried out inside a laminar air-flow chamber with full aseptic condition after complete sterilization.

3.2.5.14 Shoot initiation

The sterilized buds were cultured on MS medium with different hormonal combinations of BAP (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/L), NAA (0.5, 1.0 and 2.0 mg/L) and Kinetin (0.5, 1.0 and 2.0 mg/L)) for shoot initiation, shoot multiplication and root initiation of TCP 2 (*Curcuma longa*) whereas for *Curcuma caesia* separate combinations of BAP (0.5, 1.0, 2.0, 3.0 and 4.0 mg/L), Kinetin (0.5 mg/L) and IAA (0.25, 0.5, 0.75 and 1.0 mg/L)) were used.

3.2.5.15 Root induction

The individual shoots separated from multiple shoots, having the length of 3-5 cm long were transferred on rooting media containing different hormonal supplementations (BAP (1.0 mg/L), NAA (0.5 mg/L), Kinetin (0.5 mg/L) and IBA (0.5, 1.0, 1.5 and 2.0 mg/L)) for TCP 2 (*Curcuma longa*) whereas for *Curcuma caesia* (BAP (1.0 mg/L), IAA (0.5 mg/L), Kinetin (0.5 mg/L) and IBA (0.5, 1.0, 1.5 and 2.0 mg/L)) were used. Ten to fifteen cultures were used per treatment and each treatment of rooting experiment was repeated thrice.

3.2.5.16 Hardening and transplanting

Plantlets with well-developed roots were removed from the medium, then washed thoroughly under running tap water to remove adhering solid MS medium,

and transplanted to plastic pots containing 1:3 mixture of sterilized soilrite and sand. The plastic pots were then kept in a 50% shaded net house. The plants were frequently watered to keep high level of humidity. The fully acclimatized plants were then transferred to the field.

3.2.5.17 Callus induction and regeneration

The induction of callus was pursued by inoculating leaf tissue (blade discs, sheath base, sheath top) on a modified MS medium containing kinetin (1.0, 2.0 mg/L), 2,4-D (0.5, 1.0, 2.0 mg/L), myo-inositol (100 mg/L), sucrose (30 g/L), and agar (8.0 g/L) as solidifying agent. The inoculated materials were cultured in growth room at $25 \pm 2^\circ\text{C}$ under a photoperiod of 16/8 hours light/dark conditions. Regeneration of plantlets from callus was successfully conducted in MS medium supplemented with a combination of 2.0 mg/L BAP and 1.0 mg/L Kinetin.

3.3 Statistical analysis

3.3.1 Analysis of Variance

Analysis of Variance for factorial randomized block design was carried out as per the procedure suggested by Panse and Sukhatme (1967).

3.3.2 Phenotypic, genotypic and environmental coefficient of variation

Genotypic, phenotypic and environmental coefficients of variance were estimated according to Burton and Devane (1953).

$$\text{Phenotypic coefficient of variance (PCV)} = \frac{\sqrt{\sigma^2_p}}{\bar{X}} \times 100$$

$$\text{Genotypic coefficient of variance (GCV)} = \frac{\sqrt{\sigma^2_g}}{\bar{X}} \times 100$$

$$\text{Environmental coefficient of variance (ECV)} = \frac{\sqrt{\sigma^2_e}}{\bar{X}} \times 100$$

Where

$$\sqrt{\sigma^2_g} = \text{Genotypic variance} = \frac{\text{Treatment MSS} - \text{Error MSS}}{r}$$

$$\sqrt{\sigma^2_e} = \text{Environmental variance} = \text{Error mean sum of squares}$$

$$\sqrt{\sigma^2_p} = \text{Phenotypic variance} = \sigma^2_g + \sigma^2_e$$

$$\bar{X} = \text{General mean of the character}$$

$$r = \text{number of replications.}$$

3.3.3 Heritability

Heritability was estimated according to Burton and Devane (1953) and Allard (1960).

$$\text{Heritability } (h^2) = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where, σ^2_p = Phenotypic variance

σ^2_g = Genotypic variance

3.3.4 Expected genetic advance

Expected genetic advance (GA) was calculated as per formula given by Burton and Devane (1953) and Johnson *et al.* (1955).

$$\text{Expected genetic advance (GA)} = h^2 \times \sigma_p \times K$$

Where, h^2 = Heritability in broad sense

σ_p = Phenotypic standard deviation

K = Selection intensity, the value of which is 2.06 at 5% individual selection.

3.3.5 Genetic advance mean (GAM): It was calculated in percent.

$$\text{GAM} = \frac{GA}{\bar{x}} \times 100$$

Where, $\bar{x}$ = mean of the character

3.3.6 Genotypic and phenotypic correlation coefficients

Genotypic and Phenotypic correlation coefficients were estimated to determine the association between various character pairs. It was suggested by AL-Jibourie *et al.* (1958).

$$\text{Genotypic correlation} = \frac{\text{Cov}_{xy} (G)}{\sqrt{V_x (G) \times V_y (G)}}$$

$$\text{Phenotypic correlation} = r_{xy} (P) = \frac{\text{Cov}_{xy} (P)}{\sqrt{V_x (P) \times V_y (P)}}$$

Where,

$\text{Cov}_{xy} (G)$ = Genotypic covariance between x and y

$V_x (G)$ = Genotypic variance of character 'X'

$V_y (G)$ = Genotypic variance of character 'Y'

$\text{Cov}_{xy}(P)$ = Phenotypic covariance between x and y

$V_x(P)$ = Phenotypic variance of character 'X'

$V_y(P)$ = Phenotypic variance of character 'Y'

3.3.7 Path coefficient analysis

Path coefficient analysis was carried out following Dewey and Lu (1959) to know the direct and indirect effects of the important quantitative traits.

$$r_{1y} = P_{1y} + r_{12} P_{2y} + r_{13} P_{3y} + \dots + r_{1n} P_{ny}$$

$$r_{2y} = P_{2y} + r_{21} P_{1y} + r_{23} P_{3y} + \dots + r_{2n} P_{ny}$$

⋮

⋮

$$r_{ny} = P_{ny} + r_{n1} P_{1y} + r_{n2} P_{2y} + \dots + r_{nn} P_{ny}$$

Where,

r_{1y} to r_{ny} = Coefficient of correlation between casual factor 1 to n and dependent character Y.

r_{12} to r_{nn-1} = Coefficient of correlation between all possible combinations of casual factors.

P_{1y} to P_{ny} = Effect of characters 1 to n on the characters Y.

The effect of residual factor (z) which measures the contribution of rest of the characters obtained as-

$$P_{zy} = \sqrt{1 - R^2}$$

Where, $R^2 = \sum_{i=1}^n P_{1y} + 2 \sum_{1 < j} P_{1y} P_{jy} r_{ij}$

R^2 = Coefficient of multiple determinations

3.3.8 Genetic divergence

Mahalanobis D^2 statistics was used for assessing the genetic divergence between turmeric germplasms (Mahalanobis, 1936). It was also used in cluster analysis and classification techniques. The D^2 values were calculated as the sum of squares of differences between the pairs of corresponding uncorrelated values of any

two genotypes, described by Rao (1952). A total of $\frac{n(n-1)}{2}$ D^2 values were calculated.

Where n = number of genotypes.

3.3.9 Inter and intra cluster distances

The Inter and intra-cluster distances were calculated by the equation suggested by Singh and Chaudhary (1985).

$$\text{Inter-cluster distance} = \sqrt{D^2_{ij}/n_i n_j}$$

Where, D^2_{ij} is the sum of distances between all possible combinations ($n_i n_j$) of the genotypes included in the clusters 'i' and 'j'.

n_i = number of genotypes in the cluster 'i'

n_j = number of genotypes in the cluster 'j'

$$\text{Intra-cluster distance} = \sqrt{D^2_i/n}$$

Where, D^2_i is the sum of D^2 values between all possible combinations of the genotypes included in cluster 'i'.

3.3.10 Biochemical analysis

Statistical analyses of all biochemical procedures (quantitative estimation of curcumin content, total phenol, iron, crude fat, total protein, color content, antioxidant assay, antioxidant enzyme assay, pH measurements) were done with Microsoft Excel 2018. Principal component analysis was analyzed by SPSS 17 whereas heat map was analyzed by Heml 1.0-Heatmap Illustrator.

3.3.11 Statistical analysis of micropropagation technique

The results were subjected to statistical analysis by using one way Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT; Duncan, 1955) to determine the significant variation among various phytohormones on shoot multiplication, rooting response and induction of callus of both TCP 2 (*Curcuma longa*) and *Curcuma caesia* in MS culture medium.

Results and Discussion

The present research programme entitled “Biochemical characterization of turmeric (*Curcuma longa* L.) germplasms and standardization of protocol for micropropagation of selected cultivars” consisted of three objectives. The results of the experiments carried out under all three objectives have been presented in the subsequent sections of this chapter.

4.1 Physico-chemical analysis of soil

The physico-chemical properties of soil of the experimental field as analyzed before the start of the field study have been summarized in Table 4.1 below.

Table 4.1: Physico-chemical properties of soil of the field taken for experiment

Parameter	Value*
pH	5.72
Organic carbon (%)	0.87
Available Nitrogen (kg/ha)	170.16
Available Phosphorus (kg/ha)	18.29
Available Potassium (kg/ha)	120.45

* Average of three replicates

The soil of experimental field was acidic in nature with pH 5.72. The organic carbon as estimated was 0.87%, available nitrogen 170.16 kg/ha, available phosphorus 18.29 kg/ha and available potassium 120.45 kg/ha.

4.2 Scoring of collected genotypes of turmeric

The first objective of the present programme was scoring of collected genotypes of turmeric based on statistical analysis of some phenotypic characters and biochemical parameters. A total of sixty genotypes were collected and their curcumin content was estimated by UV-VIS spectroscopic method (Jansirani *et al.*, 2014) as presented in Table 4.2.

Table 4.2: Curcumin content in collected genotypes of turmeric

Sl. No.	Genotype	Percent curcumin (mean* $\pm$ SE)	Sl. No.	Genotype	Percent curcumin (mean* $\pm$ SE)	Sl. No.	Genotype	Percent curcumin (mean* $\pm$ SE)
1	TCP 2	5.12 $\pm$ 0.018	21	TCP 93	1.12 $\pm$ 0.019	41	TCP 194	2.27 $\pm$ 0.011
2	TCP 07	0.15 $\pm$ 0.011	22	TCP 94	0.42 $\pm$ 0.011	42	TCP 195	5.04 $\pm$ 0.007
3	TCP 11	3.07 $\pm$ 0.011	23	TCP 96	2.58 $\pm$ 0.007	43	TCP 196	3.58 $\pm$ 0.012
4	TCP 12	2.21 $\pm$ 0.014	24	TCP 100	0.17 $\pm$ 0.007	44	TCP 198	0.41 $\pm$ 0.015
5	TCP 23	1.09 $\pm$ 0.007	25	TCP 101	1.08 $\pm$ 0.007	45	TCP 200	4.08 $\pm$ 0.018
6	TCP 24	1.08 $\pm$ 0.007	26	TCP 109	0.10 $\pm$ 0.014	46	TCP 202	1.93 $\pm$ 0.014
7	TCP 34	0.45 $\pm$ 0.007	27	TCP 114	0.21 $\pm$ 0.007	47	TCP 209	1.10 $\pm$ 0.019
8	TCP 37	1.09 $\pm$ 0.012	28	TCP 137	1.81 $\pm$ 0.011	48	TCP 218	0.12 $\pm$ 0.011
9	TCP 40	1.62 $\pm$ 0.011	29	TCP 138	0.14 $\pm$ 0.011	49	TCP 221	0.21 $\pm$ 0.007
10	TCP 50	2.54 $\pm$ 0.007	30	TCP 139	1.64 $\pm$ 0.014	50	TCP 222	2.36 $\pm$ 0.014
11	TCP 54	2.86 $\pm$ 0.018	31	TCP 152	0.36 $\pm$ 0.011	51	TCP 224	1.54 $\pm$ 0.011
12	TCP 57	2.60 $\pm$ 0.008	32	TCP 153	0.13 $\pm$ 0.007	52	TCP 225	0.44 $\pm$ 0.015
13	TCP 58	1.11 $\pm$ 0.018	33	TCP 154	0.14 $\pm$ 0.007	53	TCP 227	2.51 $\pm$ 0.011
14	TCP 62	0.12 $\pm$ 0.007	34	TCP 168	0.13 $\pm$ 0.004	54	TCP 235	1.05 $\pm$ 0.012
15	TCP 63	0.32 $\pm$ 0.007	35	TCP 173	0.34 $\pm$ 0.008	55	TCP 240	2.16 $\pm$ 0.014
16	TCP 64	1.87 $\pm$ 0.014	36	TCP 175	1.42 $\pm$ 0.011	56	TCP 242	3.14 $\pm$ 0.014
17	TCP 67	4.89 $\pm$ 0.014	37	TCP 178	3.05 $\pm$ 0.018	57	TCP 244	3.92 $\pm$ 0.018
18	TCP 84	1.71 $\pm$ 0.014	38	TCP 181	3.00 $\pm$ 0.015	58	TCP 247	2.42 $\pm$ 0.011
19	TCP 87	0.15 $\pm$ 0.007	39	TCP 187	1.05 $\pm$ 0.007	59	TCP 253	0.12 $\pm$ 0.007
20	TCP 90	1.03 $\pm$ 0.019	40	TCP 190	1.04 $\pm$ 0.012	60	TCP 276	0.37 $\pm$ 0.007

*Average of three replicates

4.2.1 Grouping of collected genotypes

The genotypes were divided into three groups namely Group I with low (0.10-1.00%) curcumin content, Group II with medium (1.01-2.50%) curcumin content and Group III with high (2.51-5.50%) curcumin content and best 15 (fifteen) genotypes from each group were selected based on their curcumin content and were used for all subsequent studies. The list of selected genotypes along with their curcumin content has been presented in Table 4.3.

Among the selected genotypes, TCP 2 under Group III contained the highest curcumin content (5.12%) whereas lowest curcumin content was recorded both in TCP 154 and TCP 138 (0.14%) under Group I with 36.57 folds variation with respect to TCP 2 of Group III.

Table 4.3: Curcumin content in three groups* of selected genotypes of turmeric

Sl. No.	Genotype Group I	Percent curcumin (mean [#] ± SE)	Sl. No.	Genotype Group II	Percent curcumin (mean [#] ± SE)	Sl. No.	Genotype Group III	Percent curcumin (mean [#] ± SE)
1	TCP 154	0.14 ± 0.007	1	TCP 58	1.11 ± 0.018	1	TCP 227	2.51 ± 0.011
2	TCP 138	0.14 ± 0.011	2	TCP 93	1.12 ± 0.019	2	TCP 50	2.54 ± 0.007
3	TCP 07	0.15 ± 0.011	3	TCP 175	1.42 ± 0.011	3	TCP 96	2.58 ± 0.007
4	TCP 87	0.15 ± 0.007	4	TCP 224	1.54 ± 0.011	4	TCP 57	2.60 ± 0.008
5	TCP 100	0.17 ± 0.007	5	TCP 40	1.62 ± 0.011	5	TCP 54	2.86 ± 0.018
6	TCP 114	0.21 ± 0.007	6	TCP 139	1.64 ± 0.014	6	TCP 181	3.00 ± 0.015
7	TCP 221	0.21 ± 0.007	7	TCP 84	1.71 ± 0.014	7	TCP 178	3.05 ± 0.018
8	TCP 63	0.32 ± 0.007	8	TCP 137	1.81 ± 0.011	8	TCP 11	3.07 ± 0.011
9	TCP 173	0.34 ± 0.008	9	TCP 64	1.87 ± 0.014	9	TCP 242	3.14 ± 0.014
10	TCP 152	0.36 ± 0.011	10	TCP 202	1.93 ± 0.014	10	TCP 196	3.58 ± 0.012
11	TCP 276	0.37 ± 0.007	11	TCP 240	2.16 ± 0.014	11	TCP 244	3.92 ± 0.018
12	TCP 198	0.41 ± 0.015	12	TCP 12	2.21 ± 0.014	12	TCP 200	4.08 ± 0.018
13	TCP 94	0.42 ± 0.011	13	TCP 194	2.27 ± 0.011	13	TCP 67	4.89 ± 0.014
14	TCP 225	0.44 ± 0.015	14	TCP 222	2.36 ± 0.014	14	TCP 195	5.04 ± 0.007
15	TCP 34	0.45 ± 0.007	15	TCP 247	2.42 ± 0.011	15	TCP 2	5.12 ± 0.018

*Group I: Low curcumin content (0.10-1.00%), Group II: Medium curcumin content (1.01-2.50%),

Group III: High curcumin content (2.51-5.50%)

[#]Average of three replicates

4.2.2 Phenotypic and biochemical characters study of turmeric germplasms

The scoring of the selected genotypes of turmeric was done by statistical analysis of the results of three years (2015-16 denoted as Y1, 2016-17 as Y2 and 2017-18 as Y3) study of seven phenotypic characters namely plant height, number of tillers per plant, number of leaves per plant, leaf length, leaf breadth, clump weight and yield and two biochemical characters namely curcumin and iron content in the rhizomes. The data recorded for the above parameters and results of statistical analysis are summarized in the subsequent sections.

4.2.2.1 Plant height and number of tillers per plant

The pooled mean data as given in Table 4.4 indicated distinct variation both in plant height (102.27–167.92 cm) and number of tillers per plant (1.78–3.95/plant) among the genotypes. Highest plant height was observed in TCP 181 (167.92 cm)

and lowest was recorded in TCP 221 (102.27 cm) with an average of 138.74 cm. The maximum tiller number was recorded in TCP 173 (3.95/plant) and lowest was observed in TCP 34 (1.78/plant) with an average of 2.52/plant. The results of the present study showed conformity with those of Singh *et al.* (2000) and Datta *et al.* (2001).

4.2.2.2 Number of leaves per plant and leaf length

From the results (pooled mean data, Table 4.5) significant variation was observed both in number of leaves per plant (5.02 – 7.94) and leaf length (48.72 – 71.93 cm) among the studied genotypes. Lowest number of leaves was observed in TCP 50 (5.02/plant) and highest was recorded in TCP 12 (7.94/plant) with an average of 6.75/plant. The maximum leaf length was recorded in TCP 194 (71.93 cm) and lowest was observed in TCP 100 (48.72 cm) with an average of 61.66 cm. The findings of present study were in agreement with those of Datta *et al.* (2001) and Anon. (2010).

4.2.2.3 Leaf breadth and rhizome weight per plant

There was notable variation both in leaf breadth (12.20 – 17.08 cm) and clump weight (220.45 – 801.53 g) among the genotypes explored as revealed from the pooled mean data (Table 4.6). TCP 227 recorded lowest leaf breadth (12.20 cm) whereas TCP 54 recorded highest leaf breadth (17.08 cm) with an average of 14.50 cm. TCP 198 showed maximum rhizome weight (801.53 g) and lowest was observed in TCP 240 (220.45 g) with an average of 417.69 g. The results of the present study were in conformity with those of Datta *et al.* (2001) and Anon. (2010).

4.2.2.4 Yield

Significant variation in yield (12.52 – 38.69 ton/ha) among the genotypes under consideration was unveiled as revealed from the pooled mean data (Table 4.7). TCP 154 recorded highest yield (38.69 ton/ha) whereas TCP 34 recorded lowest yield (12.52 ton/ha) with an average of 20.72 ton/ha. The findings of present study were in agreement with those of Singh *et al.* (2000) and Anon. (2010).

Table 4.5: Number of leaves per plant and leaf length of the selected genotypes

Group	Genotype	No. of leaves* per plant				Leaf length* (cm)			
		Y1	Y2	Y3	Pooled mean	Y1	Y2	Y3	Pooled mean
Low	TCP 154	5.67	7.00	5.77	6.15	55.66	57.67	57.25	56.86
	TCP 138	4.33	8.00	5.80	6.04	47.67	70.67	47.15	55.16
	TCP 7	5.80	7.67	6.97	6.81	46.67	68.00	53.82	56.16
	TCP 87	8.00	7.67	7.17	7.61	50.67	53.33	68.09	57.36
	TCP 100	5.67	6.67	6.03	6.12	41.00	58.67	46.49	48.72
	TCP 114	6.67	8.33	7.40	7.47	51.00	68.33	51.35	56.89
	TCP 221	4.66	8.00	6.63	6.43	50.34	62.00	40.15	50.83
	TCP 63	3.00	6.33	8.17	5.83	48.00	74.33	68.32	63.55
	TCP 173	3.83	8.33	5.77	5.98	54.67	73.00	41.33	56.33
	TCP 152	6.67	8.33	8.00	7.67	47.33	59.00	75.77	60.70
	TCP 276	3.33	8.00	7.30	6.21	46.00	56.33	63.25	55.19
	TCP 198	5.50	7.33	7.57	6.80	44.34	55.33	63.47	54.38
	TCP 94	4.34	8.33	7.50	6.72	56.00	78.00	71.15	68.38
	TCP 225	5.33	7.33	6.50	6.39	46.67	71.00	55.50	57.72
	TCP 34	6.67	6.67	7.53	6.96	50.33	70.67	75.26	65.42
Medium	TCP 58	6.67	8.33	7.77	7.59	57.67	78.00	69.52	68.40
	TCP 93	4.37	7.00	8.33	6.57	55.33	71.33	74.56	67.07
	TCP 175	5.67	7.33	8.13	7.04	54.34	57.67	79.74	63.92
	TCP 224	6.00	7.67	7.03	6.90	53.33	62.00	50.80	55.38
	TCP 40	7.00	3.00	7.83	5.94	52.00	53.33	66.92	57.42
	TCP 139	5.00	7.67	8.17	6.95	46.00	68.00	75.21	63.07
	TCP 84	4.00	7.33	7.53	6.29	57.33	58.67	73.89	63.30
	TCP 137	6.00	7.00	8.90	7.30	57.00	56.33	69.66	61.00
	TCP 64	5.67	7.67	7.57	6.97	52.67	68.33	67.59	62.86
	TCP 202	6.67	7.67	8.03	7.46	55.33	70.67	69.52	65.17
	TCP 240	4.67	7.00	8.00	6.56	52.33	70.33	53.05	58.57
	TCP 12	6.67	8.33	8.83	7.94	46.67	59.00	67.94	57.87
	TCP 194	7.00	8.33	6.00	7.11	57.67	75.67	82.45	71.93
	TCP 222	4.33	7.67	7.27	6.42	54.33	85.67	57.55	65.85
	TCP 247	6.67	8.00	8.23	7.63	45.00	56.33	46.15	49.16
High	TCP 227	8.00	7.67	6.90	7.52	48.00	78.00	55.12	60.37
	TCP 50	5.67	2.67	6.73	5.02	55.33	64.67	88.25	69.42
	TCP 96	8.33	6.67	6.60	7.20	56.33	61.67	96.18	71.39
	TCP 57	5.00	6.67	7.63	6.43	56.00	82.33	65.20	67.84
	TCP 54	5.67	7.67	9.13	7.49	53.67	74.00	73.55	67.07
	TCP 181	6.00	7.67	7.50	7.06	51.33	73.00	87.71	70.68
	TCP 178	5.67	7.00	7.37	6.68	52.67	62.33	95.61	70.20
	TCP 11	5.33	6.67	6.93	6.31	52.35	78.00	75.56	68.64
	TCP 242	6.00	7.33	6.60	6.64	45.67	74.67	70.31	63.55
	TCP 196	5.67	8.00	6.87	6.85	51.67	67.33	72.42	63.81
	TCP 244	4.67	7.67	5.47	5.94	56.33	67.67	63.84	62.61
	TCP 200	5.67	7.33	7.63	6.88	49.33	56.33	67.81	57.82
	TCP 67	2.67	7.33	7.87	5.96	51.67	55.33	76.10	61.03
	TCP 195	4.67	8.00	6.60	6.42	53.33	74.00	84.81	70.71
	TCP 2	6.00	7.68	8.23	7.30	54.33	60.05	49.84	54.74
	SEm (±)	0.97	0.44	0.46	0.63	4.47	1.59	2.79	5.20
	CD (P = 0.05)	2.72	1.23	1.30	1.78	12.57	4.46	7.83	14.62
	CV (%)	30.05	10.36	10.97	16.30	15.02	4.12	7.23	14.62
Y1: 2015-2016, Y2: 2016-2017, Y3: 2017-2018; *Average of three replicates									

Table 4.7: Yield of the selected genotypes

Group	Genotype	Yield* (ton/ha)			
		Y1	Y2	Y3	Pooled mean
Low	TCP 154	37.39	29.77	48.90	38.69
	TCP 138	8.55	20.81	30.28	19.88
	TCP 7	14.58	20.64	20.49	18.57
	TCP 87	16.19	15.98	20.31	17.49
	TCP 100	18.10	30.80	24.38	24.43
	TCP 114	23.14	25.14	20.06	22.78
	TCP 221	13.48	23.73	14.42	17.21
	TCP 63	11.75	21.33	26.37	19.82
	TCP 173	12.08	36.55	47.19	31.94
	TCP 152	18.56	23.33	47.63	29.84
	TCP 276	21.12	21.72	32.01	24.95
	TCP 198	15.48	28.56	43.09	29.04
	TCP 94	26.00	28.24	34.25	29.50
	TCP 225	12.75	20.91	17.83	17.16
	TCP 34	10.45	10.86	16.24	12.52
Medium	TCP 58	25.75	34.18	44.78	34.90
	TCP 93	12.75	15.29	39.10	22.38
	TCP 175	20.69	10.64	22.03	17.79
	TCP 224	19.88	12.95	14.68	15.84
	TCP 40	25.18	33.39	24.73	27.77
	TCP 139	20.20	18.61	17.73	18.85
	TCP 84	18.54	12.87	17.24	16.22
	TCP 137	20.82	22.15	28.64	23.87
	TCP 64	18.89	14.48	27.11	20.16
	TCP 202	16.48	16.09	24.31	18.96
	TCP 240	18.26	10.18	12.88	13.77
	TCP 12	16.29	42.12	19.07	25.83
	TCP 194	17.70	24.54	12.47	18.24
	TCP 222	16.83	21.32	19.69	19.28
	TCP 247	19.80	19.39	10.21	16.47
High	TCP 227	17.90	24.55	22.43	21.63
	TCP 50	21.06	12.51	19.56	17.71
	TCP 96	13.16	18.37	16.92	16.15
	TCP 57	10.73	11.34	15.95	12.67
	TCP 54	10.06	17.70	12.37	13.38
	TCP 181	18.85	23.20	12.89	18.31
	TCP 178	21.59	32.60	28.60	27.60
	TCP 11	23.20	26.91	23.83	24.65
	TCP 242	19.38	12.87	13.12	15.12
	TCP 196	15.60	30.97	9.65	18.74
	TCP 244	15.49	15.55	13.59	14.88
	TCP 200	19.33	14.48	20.19	18.00
	TCP 67	14.79	25.34	14.83	18.32
	TCP 195	14.61	15.67	13.84	14.71
	TCP 2	13.00	17.75	16.63	15.79
	SEm (±)	3.34	4.59	3.93	3.84
	CD (P = 0.05)	9.40	12.90	11.04	10.79
	CV (%)	32.73	37.02	29.64	32.12
Y1: 2015-2016, Y2: 2016-2017, Y3: 2017-2018; *Average of three replicates					

4.2.2.5 Curcumin and iron content

The pooled mean data exhibited significant variation both in curcumin content (0.14 – 5.32%) and iron content (41.91 – 55.43 mg/100 g) among the genotypes studied (Table 4.8). The genotype TCP 195 showed highest curcumin content (5.32%) followed by TCP 2 (5.12%) while TCP 87 exhibited lowest curcumin content (0.14%). Highest iron content was observed in TCP 96 (55.43 mg/100 g) whereas TCP 221 showed its lowest value (41.91 mg/100 g). The results of the present study were in well conformity with those of Muralidharan and Ramankutty (1976) and Madhusankha *et al.* (2018).

Although mean value revealed that TCP 195 contained highest curcumin (5.32%) followed by TCP 2 (5.12%) but out of three years study the results of last two years showed TCP 2 having higher curcumin content than TCP 195. Such type of variation was observed in case of lowest curcumin content and iron content of some genotypes also. The mean value of three years study showed TCP 96 had highest iron content (55.43 mg/100 g) and TCP 221 had its lowest value (41.91 mg/100 g). But results of last two years showed TCP 195 contained highest iron (55.25 mg/100 g).

4.2.3 Variability studies

An information on genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), environmental coefficient of variation (ECV), estimates of heritability (h^2) and expected genetic advance mean (GAM) percentage in respect of yield and yield determining traits constitute the basic requirement for crop improvement programme. The analysis of variance for the selected forty five genotypes was used to evaluate the significant difference among genotypes of turmeric and this has been limited to nine key characters. The analysis of variance revealed that all the genotypes varied for all the traits under the present programme. Experimental results from GCV, PCV, ECV, h^2 and GAM for the mean of three years have been statistically analyzed and presented in Table 4.9.

Table 4.9: Variability parameters in turmeric genotypes studied

Parameter	GCV (%)	PCV (%)	ECV (%)	h^2 (%)	GAM (%)
Plant height (cm)	11.46	13.10	6.33	76.61	20.67
Tiller number/plant	14.68	24.94	20.16	34.64	17.79
Leaf number/plant	7.16	12.28	9.99	33.92	8.58
Leaf length (cm)	9.46	10.94	5.50	74.70	16.83
Leaf breadth (cm)	6.48	8.36	5.29	60.00	10.34
Rhizome weight (g/plant)	26.30	32.45	19.09	65.66	43.90
Yield (ton/ha)	26.75	32.56	18.56	67.52	45.29
Curcumin (%)	75.99	76.56	9.30	98.52	155.38
Iron (mg/100 g)	4.18	5.70	3.87	53.76	6.31

4.2.3.1 GCV, PCV and ECV analysis

It was observed from the results that GCV was highest for curcumin content (75.99) followed by yield (26.75), rhizome weight (26.30), tiller number (14.68), plant height (11.46), leaf length (9.46), leaf number (7.15), leaf breadth (6.48) whereas lowest for iron content (4.18). The highest PCV percentage was also observed for curcumin content (76.56) followed by yield (32.56), rhizome weight (32.45), tiller number (24.93), plant height (13.10), leaf number (12.28), leaf length (10.94), leaf breadth (8.36) whereas lowest was observed for iron content (5.70). In case of ECV, tiller number exhibited highest (20.16) value followed by rhizome weight (19.09), yield (18.56), leaf number (9.99), curcumin percentage (9.30), plant

height (6.33), leaf length (10.94) and leaf breadth (8.36) whereas lowest was found in iron content (3.87).

High PCV and GCV value defines that the genotypes reveal evidence of much variation among themselves with respect to morphological and biochemical traits. Lowest difference of PCV and GCV specify that genotypes do not exhibit much variation among themselves with respect to these morphological and biochemical parameters (Baye *et al.*, 2005).

From the results of the present study it was found that there was a wide range of difference for all the parameters providing the vast scope to select the desirable genotype. The difference between GCV and PCV was high in case of tiller number followed by rhizome weight and yield indicating that environmental factor affected these characters to a greater extent (Joshi *et al.*, 2018). On the other hand, very little difference was found between GCV and PCV for curcumin content followed by iron content, leaf length, leaf breadth and plant height which suggested that these characters were less dependent on environmental factor as PCV values were higher in all the cases than the GCV values indicating that environment possessed slight to moderate influence (Janaki *et al.*, 2015). The lesser the difference, lesser the influence but it is more than corresponding values of the individual parameters. Therefore, very high selection response was found in respect of curcumin content, iron content, leaf length, leaf breadth and plant height. PCV was higher than GCV value for curcumin content but their difference was less. The findings of the present study were in agreement with those of Reddy and Rao (1988) and Singh *et al.* (2007). Reddy and Rao (1988) carried out similar type of work in turmeric with 32 genotypes. They studied fifty three characters for PCV, GCV, heritability and expected GAM percentage. Among the fifty three characters, twenty five characters showed relatively high GCV, twenty one traits had medium and the rest seven parameters had low GCV. So, it could be concluded that influence of environment is less on the curcumin content.

4.2.3.2 Heritability (h^2) and GAM analysis

High estimates of heritability was recorded for curcumin content (98.52), plant height (76.61), leaf length (74.70), yield (67.52), rhizome weight (65.66) and leaf breadth (60.00) whereas low (<60.00) estimates of heritability was found for iron content (53.76), tiller number (34.64) and leaf number (33.92).

Heritability estimates the degree of variation in a phenotypic trait in a population that is due to genetic variation between individuals (Maurya *et al.*, 2018). High heritability found for different parameter suggested that large variation for phenotypic variance was imputed to genotypic variance and for this reason, selection would be assembled on the basis of phenotypic coefficient of variation. High heritability observed in the present study for the parameters under consideration indicate less influence of the environment and so there is a good scope for improvement of these parameters through direct selection (Kumar *et al.*, 2012). The findings of the present study were supported by those of Datta *et al.* (2006), Singh *et al.* (2012) and Rajyalakshmi *et al.* (2013). Rajyalakshmi *et al.* (2013) did similar type of work with 27 genotypes of turmeric and observed that magnitude of heritability ranged from 62.7 – 92.9% and GAM varied from 10.1 – 79.2%.

In case of genetic advance mean percentage, highest value was found in curcumin content (155.38) whereas much lower values were observed in yield (45.29), rhizome weight (43.90), plant height (20.67), tiller number (17.79), leaf length (16.83), leaf breadth (10.34), leaf number (8.58) and iron content (6.31).

High GCV together with high heritability and high GAM gives better information than single character alone. GAM indicates the magnitude of expected genetic gain from one cycle of selection (Hamdi *et al.*, 2003). Heritability estimates along with genetic advance mean are more useful in predicting selection of the best individual (Johnson *et al.*, 1955). High heritability along with GAM of variability parameters are predominantly controlled by additive gene action and further improvement may happen through selection (Mishra *et al.*, 2015). High values of GAM are signs of additive gene action while low values are proof of non-additive gene action (Singh and Narayanan, 1993). Therefore, heritability determines will be reliable if coincides with high GAM. Estimation of GCV with heritability gives the clear scenario of heritable variation (Burton, 1952).

From the results of high heritability (98.52) and high genetic advance mean of curcumin content (155.38) it can be predicted that there is a large scope for development through selection based on curcumin content. The results of the present study showed conformity with the works of Singh *et al.* (2012), Datta *et al.* (2006) and Rao *et al.* (2004).

In addition, selection based on the other parameters namely yield, rhizome weight, leaf breadth, leaf length and plant height may also be considered.

In the present study GCV, PCV, h^2 and GAM were highest for average curcumin content. So, keeping parity with the observations of Luiram *et al.* (2018) it can be concluded that the individual plant selection based on these characters can effectively bring about genetic improvement in turmeric.

4.2.3.3 Genotypic and phenotypic correlation coefficient

Correlation coefficient estimates the degree and direction of correlation among various parameters and finds out the component traits on which selection can be made effectively. Genotypic and phenotypic correlations are essential for building indirect selection implications between two traits. The genotypic and phenotypic correlation coefficient was restricted to nine characters in the present study. Experimental results from genotypic and phenotypic correlation for the mean of three years have been analyzed statistically and presented in Table 4.10. The results showed that genotypic correlations were higher in magnitude than phenotypic correlation values indicating strong correlation among the traits used in this study and little influence of environment. Yadav *et al.* (2006) also studied higher estimates of genotypic correlation than corresponding phenotypic correlation between yield and yield components in turmeric.

Table 4.10: Genotypic and phenotypic correlation coefficient of the selected genotypes

	PH	TN	LN	LL	LB	RW	Y	CUR	Iron
PH	0.00	0.07	0.08	0.65**	0.34*	0.11	0.38*	0.07	0.16
TN	0.05	0.00	0.05	0.24	0.02	0.23	0.19	-0.10	-0.10
LN	0.01	0.17	0.00	0.25	0.36*	-0.17	0.17	0.10	0.17
LL	0.76++	0.61+	0.44+	0.00	0.42**	-0.02	0.30*	-0.06	0.04
LB	0.25	0.04	0.64++	0.54+	0.00	0.04	0.07	0.16	0.10
RW	0.41	0.38+	-0.22	-0.05	0.35	0.00	0.32*	0.09	-0.18
Y	0.83+	0.09	0.29+	0.63+	0.12	0.50+	0.00	-0.16	0.14
CUR	0.45	-0.27	0.18	0.28	0.32	0.07	-0.26	0.00	0.41**
Iron	0.08	-0.20	0.22	-0.17	0.08	-0.26	0.18	0.78++	0.00

(PH - plant height, TN - tiller number, LN - leaf number, LL - leaf length, LB - leaf breadth, RW - rhizome weight, Y - yield, CUR- curcumin percent, Iron – Iron percent)

From the results of the present study, it was found that plant height showed significantly positive association genotypically with leaf length (0.76) and yield (0.83) but phenotypically with leaf length (0.65), leaf breadth (0.34) and yield (0.38).

Tiller number exhibited significant positive correlation genotypically with leaf length (0.61) and rhizome weight (0.38) but phenotypically no correlation with other characters. Leaf number showed significant positive association genotypically with leaf length (0.44), leaf breadth (0.64) and yield (0.29) but phenotypically with leaf breadth (0.36) only. Leaf length recorded significant positive correlation genotypically with plant height (0.76), tiller number (0.61), leaf number (0.44), leaf breadth (0.64) and yield (0.29) but phenotypically with plant height (0.65), leaf breadth (0.54) and yield (0.63) only.

Leaf breadth showed significant positive association genotypically with leaf number (0.64) and leaf length (0.54) but phenotypically with plant height (0.34), leaf number (0.36) and leaf length (0.42). Rhizome weight exhibited significant positive correlation genotypically with tiller number (0.38) and yield (0.50) but phenotypically with yield (0.32) only. Yield showed significant positive association genotypically with plant height (0.83), leaf number (0.29), leaf length (0.63) and rhizome weight (0.50) but phenotypically with plant height (0.38), leaf length (0.30) and rhizome weight (0.32) which suggested that these traits had least contribution for increasing the rhizome yield.

Curcumin recorded significant positive correlation genotypically (0.78) and phenotypically (0.41) with iron but no correlation with other characters. On the other hand, iron showed significant positive association genotypically (0.78) and phenotypically (0.41) with curcumin content.

From the results it can be concluded that increase in plant height, leaf number, leaf breadth also increases the leaf length and yield. Plant height showed highly significant positive association with rhizome yield in this study. Similar type of result was observed in the correlation study between rhizome yield with leaf width, plant height, rhizome length, rhizome width, rhizome weight, secondary fingers per rhizome and mother rhizomes per plant by Singh *et al.* (2018). They observed that green rhizome yield was significantly correlated with plant height, rhizome length, rhizome width, rhizome weight, secondary fingers per rhizome and mother rhizomes per plant at both phenotypic and genotypic level while rhizome weight showed positive significant correlation with mother rhizomes per plant at genotypic as well as phenotypic levels, respectively.

This correlation analysis suggested that tall plants having longer and prolonged leaves will be a suitable plant type for higher rhizome yield. Curcumin content was observed to have highly significant positive correlation with iron content which implies that with the increase in curcumin content, iron content will also be increased.

4.2.3.4 Path coefficient analysis

Correlation coefficient exhibited only the relationship between yield and yield components, but did not reveal any direct and indirect effects of different yield components on the rhizome yield. In this situation, path coefficient analysis is a procedure to partition the observed correlation coefficient into direct and indirect effects of yield components to provide more distinct picture of character association for formulating efficient selection strategy. The results from the pooled mean over three years have been presented in Table 4.11.

Table 4.11: Path coefficient analysis of the selected genotypes

	PH	TN	LN	LL	LB	RW	Yield	Iron
PH	0.847	-0.001	-0.003	-0.367	0.012	-0.005	0.006	0.121
TN	0.034	-0.021	0.008	-0.037	0.004	0.010	-0.046	0.009
LN	-0.034	-0.002	0.077	0.009	0.006	0.004	-0.043	-0.046
LL	0.677	-0.002	-0.002	-0.458	0.011	0.002	-0.027	0.158
LB	0.330	-0.002	0.014	-0.160	0.032	0.004	0.004	-0.042
RW	-0.085	-0.004	0.006	-0.018	0.003	0.052	-0.028	-0.126
Y	-0.034	-0.007	0.022	-0.083	-0.001	0.010	-0.150	-0.019
Iron	0.220	0.000	-0.008	-0.156	-0.003	-0.014	0.006	0.465

(PH - plant height, TN - tiller number, LN - leaf number, LL - leaf length, LB - leaf breadth, RW - rhizome weight, Y- yield, Iron - Iron percent)

Experimental results revealed that plant height exhibited significantly positive direct effect on curcumin content (0.847). Iron content also showed positive direct effect on curcumin (0.465). Leaf length showed positive direct effect on plant height (0.677) but indirect effect on curcumin content (-0.458). Most of the other characters showed significantly negligible effect on curcumin.

So, it can be concluded that plant height is the vital phenotypic character on which selection for high yield and curcumin rich genotypes can be made. Similar results that plant height determined yield potential of the genotypes for crop development in turmeric was observed by Narayanpur and Hanamashetti (2003). In

addition, curcumin rich genotypes would be high in iron content because iron exhibited positive effect on curcumin. The findings of the present study were in agreement with those of Tomar *et al.* (2005) and Singh and Ramakrishna (2014).

4.2.3.5 Distribution of turmeric genotypes into clusters

Mahalanobis D^2 analysis (Mahalanobis, 1936) gives a suitable technique to estimate the divergence present in the genotypes and also helps in selection of the genetically different genotype. The experimental findings relate to the distribution of turmeric genotypes into clusters based on Mahalanobis D^2 Values. These experimental results for the pooled mean over three years (2015-16, 2016-17 and 2017-18) have been given in Table 4.12. Analysis of variance exhibited that genotypes differed from one another for all the parameters under the present programme.

Based on D^2 value, forty five turmeric genotypes were grouped into eight clusters. Cluster I comprised of highest number of genotypes (14) and contributed 31.11% to form the cluster. Cluster V contained second highest number of genotypes (11) followed by cluster VIII (8), cluster IV and VII (3) whereas lowest number of genotypes was observed for cluster II, III and VI (2). Their contribution percentages towards clustering were 24.44%, 17.78%, 6.67% and 4.44% respectively.

Table 4.12: Distribution of turmeric genotypes into clusters

Cluster	Genotype Count and Distribution (%)	Individual Genotypes
Cluster I	14 (31.11%)	TCP-198, TCP-114, TCP-63, TCP-87, TCP-221, TCP-7, TCP-152, TCP-154, TCP-138, TCP-276, TCP-94, TCP-100, TCP-173, TCP-225
Cluster II	2 (4.44%)	TCP-84, TCP-178
Cluster III	2 (4.44%)	TCP-64, TCP-242
Cluster IV	3 (6.67%)	TCP-34, TCP-202, TCP-240
Cluster V	11 (24.44%)	TCP-58, TCP-93, TCP-175, TCP-224, TCP-40, TCP-139, TCP-137, TCP-12, TCP-194, TCP-11, TCP-196
Cluster VI	2 (4.44%)	TCP-227, TCP-50
Cluster VII	3 (6.67%)	TCP-222, TCP-247, TCP-2
Cluster VIII	8 (17.78%)	TCP-96, TCP-57, TCP-54, TCP-181, TCP-244, TCP-200, TCP-67, TCP-195

This clustering pattern revealed that there was no association of genotypes. Therefore, formation of cluster with different genotypes suggested diversity among genotypes. Each cluster was distantly related with the other. Among the different clusters, cluster V was more divergent because it contained nine medium curcumin containing genotypes and two high curcumin containing genotypes. This grouping of genotypes into various clusters did not follow any specific pattern and was found independent according to their geographical area. But, there was no association between geographical and genetic diversity. The magnitude of D^2 values assured that there was significant amount of diversity in the experimental material evaluated as supported by the works of Biju *et al.* (2004) and Haralayya *et al.* (2017). The latter group did similar type of work in french bean and estimated thirty six genotypes for thirteen yield and yield attributing traits. The genotypes were grouped into six clusters on the basis of Mahalanobis D^2 analysis. Cluster I contained the highest genotypes (19) followed by cluster VI, cluster II and cluster III.

4.2.3.6 Inter and intra cluster distance

Inter and intra cluster distance is the index of genetic diversity among clusters (Verma *et al.*, 2018). Experimental findings for the pooled mean over three years (2015-16, 2016-17 and 2017-18) of inter and intra cluster distances among different clusters are presented in Table 4.13. The inter cluster average D^2 value was maximum ($D^2 = 16.07$) between cluster VI and cluster VII which indicated that the genotypes of cluster VI were far away from the genotypes of cluster VII and species occupying in these clusters to be genetically diverse. Inter cluster average D^2 value between cluster V and cluster VI ($D^2 = 11.89$), and between cluster VII and cluster VIII ($D^2 = 11.78$) also indicated significant distances. The lowest inter cluster average D^2 value was recorded between cluster II and cluster III ($D^2 = 2.74$) which suggested close association among the genotypes involved for various characters and the genotypes of these two clusters were harmonious to one another for the studied parameters.

Table 4.13: Inter and intra cluster distances

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Cluster I	6.73*	4.99	5.64	8.86	6.50	7.08	12.11	7.38
Cluster II		1.60	2.74	7.89	4.72	5.25	11.36	5.71
Cluster III			1.81	7.27	5.15	6.47	10.10	5.49
Cluster IV				8.07	9.17	11.89	7.38	9.08
Cluster V					6.17	6.12	12.62	7.10
Cluster VI						1.97	16.07	7.87
Cluster VII							3.09	11.78
Cluster VIII								7.86

*Bold value indicates intra cluster distance

On the other hand, cluster IV ($D^2 = 8.07$) was found to have maximum intra cluster average D^2 value followed by cluster VIII ($D^2 = 7.86$), cluster I ($D^2 = 6.73$), cluster V ($D^2 = 6.17$), cluster VII ($D^2 = 3.09$), cluster VI ($D^2 = 1.97$) and cluster III ($D^2 = 1.81$) whereas lowest intra cluster average D^2 value was found in cluster III ($D^2 = 1.81$). Cluster II and III suggested lesser genetic differences among the genotypes of these eight clusters. Low level of intra cluster distance revealed narrow genetic variation within the cluster.

The results showed that inter cluster distance was always larger than intra cluster distance indicating extensive genetic diversity among the genotypes of different groups and exhibiting important role in crop improvement programmes to produce population with wider variability. The findings of present study were in agreement with those of Verma *et al.* (2018) and Haralayya *et al.* (2017). Verma *et al.* (2018) did same type of work in turmeric and found that all genotypes were divided into 10 clusters and cluster III contained maximum number of genotypes.

4.2.3.7 Cluster mean values for different characters

Cluster mean values for nine characters were estimated for pooled mean over three years (2015-16, 2016-17 and 2017-18). It was observed from the results (Table 4.14) that cluster VII contained highest cluster mean values for different characters (3) such as plant height (151.24), curcumin percentage (4.79) and iron content (53.56). Cluster III and VI consisted second highest mean values of various characters (2) such as leaf number (7.53) and leaf breadth (15.62), and tiller number (2.97) and yield (27.29) respectively. Cluster V and VIII held lowest mean values for

different characters (1) such as rhizome weight (452.62) and leaf length (66.73) respectively. Cluster means for different parameters exhibited that considerable variation was present among the clusters for all the characters.

Table 4.14: Cluster mean values for different characters

	Plant height (cm)	Tiller number (/plant)	Leaf number (/plant)	Leaf length (cm)	Leaf breadth (cm)	Rhizome weight (g/plant)	Yield (ton/ha)	Curcumin (%)	Iron (mg/100 g)
Cluster I	128.74	2.61	6.45	60.34	13.89	434.32	19.07	1.66	48.33
Cluster II	128.68	2.06	6.77	59.22	14.63	305.81	19.50	1.61	48.82
Cluster III	139.39	2.24	7.53	60.98	15.62	322.28	20.35	2.06	52.26
Cluster IV	149.37	2.41	6.42	61.43	13.74	420.35	17.38	3.25	51.49
Cluster V	142.62	2.54	6.54	60.84	14.33	452.62	22.19	1.39	50.37
Cluster VI	131.84	2.97	7.31	58.81	14.33	402.82	27.29	0.31	48.20
Cluster VII	151.24	2.26	6.79	61.44	15.57	420.36	17.95	4.79	53.56
Cluster VIII	146.34	2.55	7.31	66.73	15.41	394.08	22.66	1.96	51.46

These cluster mean values could be utilized to fulfill the objective of breeding program. Similar type of work was done by Verma *et al.* (2018) in turmeric. They found that total genotypes were divided into ten different clusters on the basis of principal component analysis.

4.2.3.8 Contribution of each character towards divergence

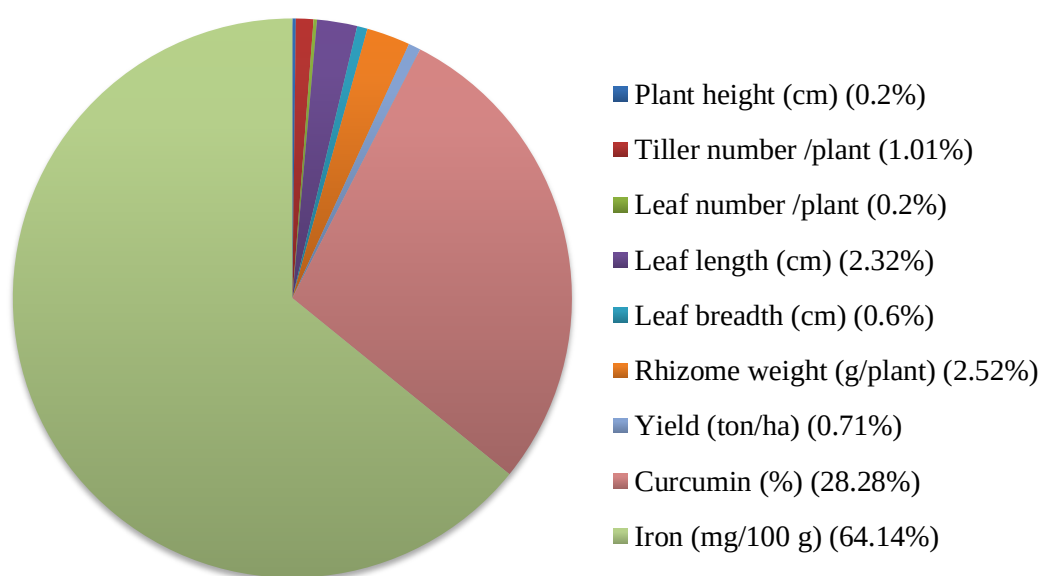
Genetic diversity is the variation of heritable characteristics present in a population of one species and serves as a way for populations to adopt in changing environment. This diversity analysis greatly helps the breeder to identify the appropriate genotypes for specific breeding objectives and also in hybridization programme. The experimental results from the pooled mean over three years of study have been given in Table 4.15.

Out of the nine characters recorded, iron content contributed maximum (64.14%) towards genetic divergence followed by curcumin percentage (28.28%), rhizome weight (2.52%), leaf length (2.32%), tiller number (1.01%), yield (0.71%), leaf breadth (0.60%) whereas leaf number (0.20%) and plant height (0.20%) contributed to the minimum extent. The percentage contribution of nine characters towards genetic divergence has been graphically presented in Fig 4.1.

Table 4.15: Contribution of each character towards divergence

Character	No. of first rank	Percent contribution
Plant height (cm)	2	0.20
Tiller number /plant	10	1.01
Leaf number /plant	2	0.20
Leaf length (cm)	23	2.32
Leaf breadth (cm)	6	0.60
Rhizome weight (g/plant)	25	2.52
Yield (Ton/ha)	7	0.71
Curcumin (%)	280	28.28
Iron (mg/100 g)	635	64.14

Among the different characters iron content showed first rank (635) towards divergence followed by curcumin percentage (280), rhizome weight (25), leaf length (23), tiller number (10), yield (7), leaf breadth (6) leaf number (2) and plant height (2). These parameters are essential from the point of view of genetic diversity analysis. The distinctive range in the morphological and biochemical characters across genotypes recorded in this research meant that each genotype might be distinguished individually (Prajapati *et al.*, 2014). The findings of the present study were in agreement with those of Haralayya *et al.* (2017) and Verma *et al.* (2018).

**Fig 4.1:** Graphical presentation of percentage contribution of studied parameters towards genetic divergence

4.2.3.9 Principal Component Analysis

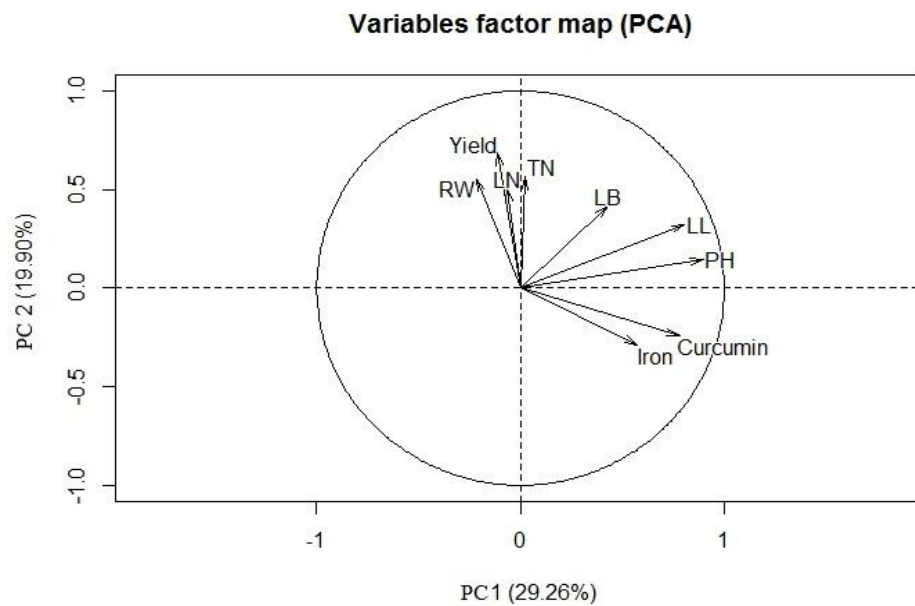
Principal component analysis (PCA) is a technique that is useful for the classification and compression of data. The purpose is to reduce the dimensionality of a data set (sample) by finding a new set of variables, smaller than the original set of variables which represents the direction of the maximum variance of the given data. This analysis is used to identify the most significant variables contributing to the total variation in diversity analysis and also gives useful information about the differences between genotype collections. PCA and cluster analysis represent the complex relationship between genotypes (Rezai and Frey, 1990). The findings of the analysis of the pooled mean data over three years have been given in Table 4.16.

Table 4.16: Variation among turmeric germplasms accounted for first four principal components

Traits	PC 1	PC 2	PC 3	PC 4
Eigenvalues	2.63	1.79	1.15	0.98
Variance (%)	29.26	19.90	12.72	10.84
Cumulative variance (%)	29.26	49.16	61.88	72.72
	Eigenvectors			
Plant height (cm)	0.55	-0.11	0.14	-0.08
Tiller number (/plant)	0.02	-0.42	-0.35	-0.24
Leaf number (/plant)	-0.04	-0.38	-0.04	0.80
Leaf length (cm)	0.50	-0.24	-0.01	-0.19
Leaf breadth (cm)	0.26	-0.31	0.54	0.18
Rhizome weight (g/ plant)	-0.13	-0.41	0.22	-0.46
Yield (Ton/ ha)	-0.07	-0.51	-0.46	0.03
Curcumin (%)	0.48	0.18	-0.04	0.12
Iron (mg/100 g)	0.35	0.21	-0.55	0.03

The four principal components (PCs) with eigenvalues near about one accounted for 72.72% of the variability among 45 turmeric germplasms. First principal component (PC 1) accounted for 29.26% of the total variation whereas second principal component (PC 2) contributed 19.90% of total variance. Third principal component (PC 3) contributed around 12.72% of the variability present among the accessions for the characters whereas fourth principal component (PC 4) accounted for 10.84% of the total variation. The phenotypic characters which were contributed more positively to PC 1 included plant height (0.55), leaf length (0.50), leaf breadth (0.26), curcumin percentage (0.48) and iron content (0.35) whereas PC 2 was associated positively with curcumin percentage (0.18) and iron content (0.21) of

the turmeric genotypes. PC 3 was correlated positively with plant height (0.14), leaf breadth (0.54) and rhizome weight (0.22) whereas PC 4 was contributed positively with leaf number (0.80), leaf breadth (0.18) and curcumin percentage (0.12). This analysis is a beneficial method to group turmeric genotypes that may help in crop improvement programmes. The findings of the present study were in agreement with those of Roy *et al.* (2011) and Verma *et al.* (2018).



(PH - plant height, TN - tiller number, LN - leaf number, LL - leaf length, LB - leaf breadth, RW - rhizome weight, Iron – Iron percent, Curcumin - curcumin percent)

Fig. 4.2 Variables factor map of first two principal components showing the contribution of various traits based on mean values in the separation of various genotypes of turmeric

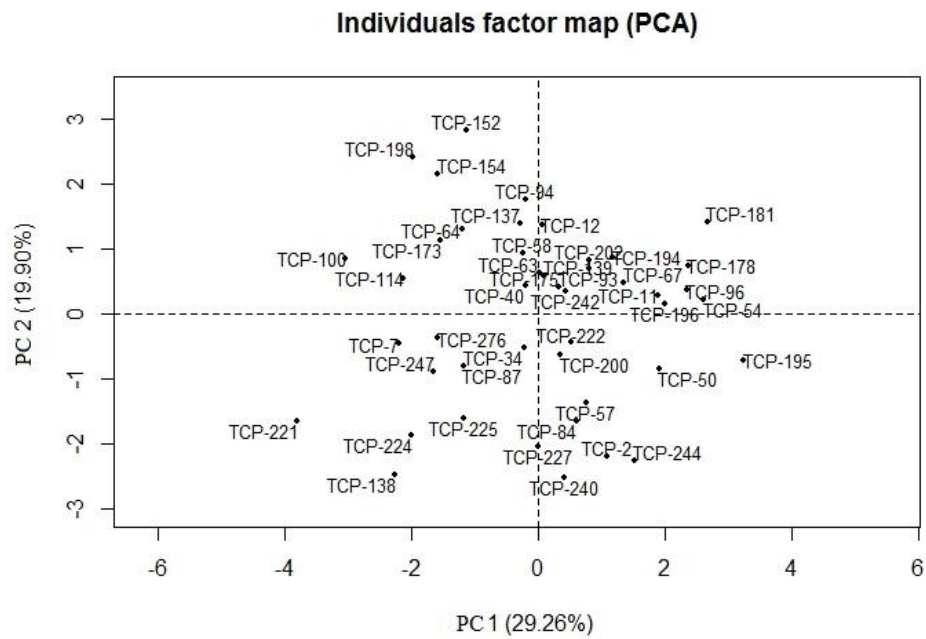


Fig. 4.3 Individuals factor map of first two principal components based on mean values of nine quantitative traits in 45 genotypes of turmeric

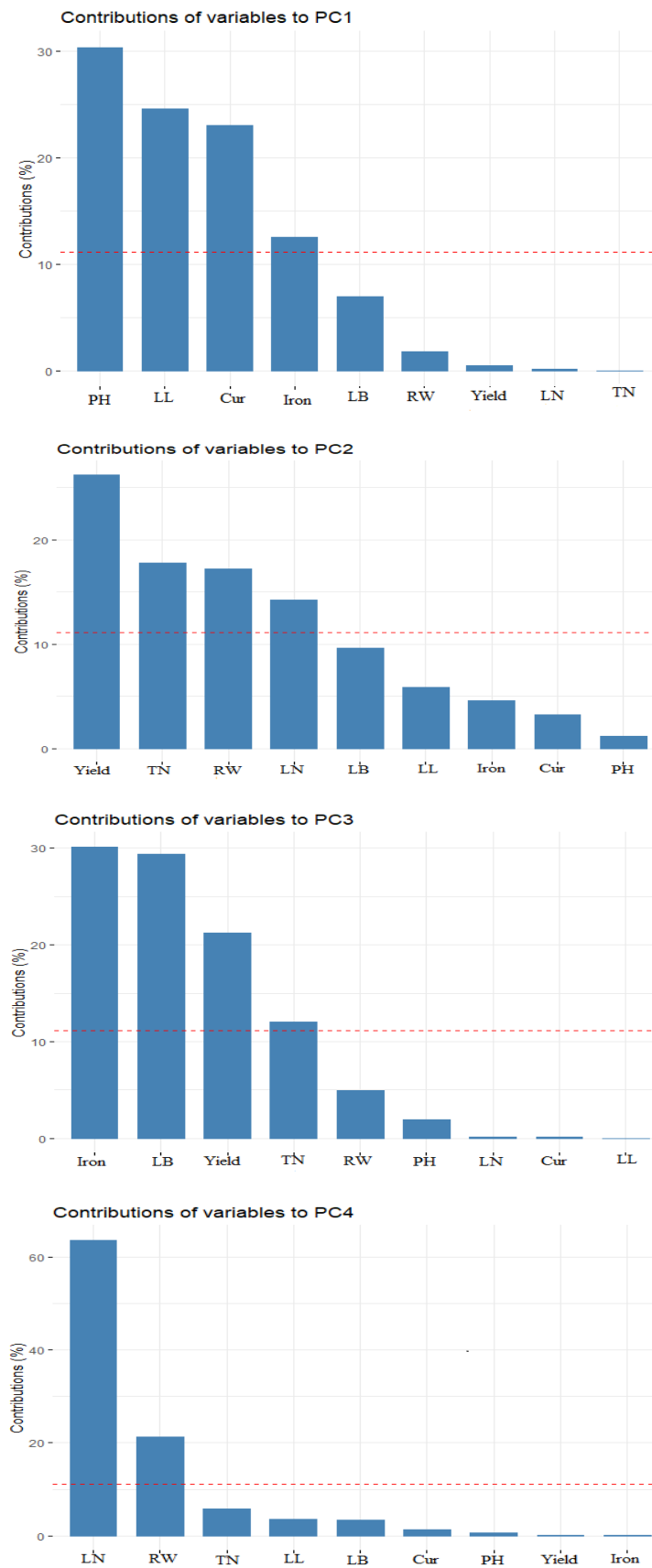


Fig. 4.4 Contribution of different variables to PC1, PC2, PC3 and PC4

The relationship among different traits was presented in Fig. 4.2 and that among the genotypes in Fig. 4.3 whereas contributions of different variables to PC 1, PC 2, PC 3 and PC 4 were shown in Fig. 4.4. The figures showed clearly about the genotypes and traits. All clusters revealed clear separation on the biplot (Fig. 4.2). Curcumin and iron content were positively correlated which was also found in genotypic and phenotypic correlation. Therefore, these occupied the lower right corner of the biplot. On the other hand, plant height, leaf length and leaf breadth were positively associated with one another but no correlation with curcumin and iron content. So, these were plotted on the upper right corner of the biplot. Similarly, rhizome weight, leaf number and yield were correlated with each other and these were plotted on the upper left corner of the biplot. Plant height occupied far away from the center among the all parameters. So, plant height was the most important morphological character for increasing the rhizome yield of turmeric.

4.3 Estimation of quality related biochemical parameters

The biochemical parameters those were estimated under the second objective included quantitative estimation of curcumin, rhizome colour, total phenol, protein, iron and crude fat; assay of antioxidant activity by ABTS, DPPH, TAC and CUPRAC methods; assay of antioxidant enzyme activity namely catalase, peroxidase and superoxide dismutase; and measurement of pH changes of turmeric powder. All the biochemical parameters except curcumin and iron content were measured in one year (2017-18) only. The results found for all the above parameters have been presented in the following sections.

4.3.1 Curcumin content in the selected genotypes

Curcumin is the basic coloring substance under polyphenolic chemical group present in *Curcuma longa* and primarily responsible for the diverse medicinal and biological properties of turmeric. The curcumin content in the dried rhizomes of sixty genotypes of turmeric collected from AICRP on Spices, Pundibari Centre were estimated by UV-VIS spectrophotometric method, results of which are recorded in Table 4.2. Based on curcumin content the genotypes were classified into three groups namely Group I with low curcumin content (0.10-1.00%), Group II with medium curcumin content (1.01-2.50%) and Group III with high curcumin content (2.51-5.50%). Best fifteen genotypes from each group were selected (Table 4.3) and used for further studies.

Among the three groups, genotypes under Group III possessed 2.51-5.12% curcumin where the variety TCP2 had the highest curcumin content (5.12%). This was followed by TCP 195 (5.04%), TCP 67 (4.89%), TCP 200 (4.08%) and TCP 244 (3.92%) whereas lowest curcumin content was recorded in TCP-154 (0.14%) and TCP-138 (0.14%) with 36.57 fold variation with respect to the highest one. In one earlier study curcumin content estimated in freshly harvested rhizomes of 38 varieties of *C. longa* and *C. aromatica* ranged from 2.5 to 8.1% (Mathai, 1976) and in another study five turmeric genotypes exhibited curcumin content from 3.76 to 5.05% (Madhusankha *et al.*, 2018). So, the results of the present study were analogous to the earlier works.

4.3.2 Colour content estimation

Colour is one of the most important attributes of turmeric rhizome quality and hence considerable attention has been given for its characterization and measurement. Turmeric is harvested when the aerial part of this plant senesces after flowering and rhizomes develop an intense yellow colour indicating the abundant presence of desired pigment curcumin. The colour of mature rhizome of turmeric is largely due to the presence of the polyphenolic pigment curcumin. It possesses excellent thermal stability and is resistant to pH until the system is quite alkaline. It forms complexes with protein, is highly resistant to oxidation and is believed to have antioxidant properties more effective than synthetic antioxidants like butylated hydroxytoluene (BHT). The presence of cations probably changes the colour to orange-brown. In acidic systems, the colour is lemon-yellow with a characteristic green fluorescence. As the pH increases, the greenish tint diminishes and ultimately the hue becomes orange.

Although several studies have been carried out to characterize the biological property of turmeric extracts but considerable scientific gap is found to exist between the biochemical basis of colour formation with other quality related parameters.

The first and most important biochemical parameter in turmeric quality assessment is curcumin content of the rhizome. Based on the curcumin content the colour of the rhizome varies from deep orange to light yellow. In the present study the L^* value ranged from 44.31 to 65.63 whereas a^* value varied from 8.69 to 22.73 (Table 4.17). The L^* value as observed was highest in TCP 154 (65.63) and lowest was recorded in TCP 194 (44.31) with 1.48 folds variation with respect to the highest one. The genotype TCP 2 showed highest a^* value (22.73) whereas lowest was recorded in TCP 154 (8.69) with 2.61 folds variation with respect to the highest one. The b^* value varied from 38.26 to 52.23 while hue angle (h°) ranged from 63.40 to 80.36. The b^* value was found highest in TCP 221 (52.23) whereas lowest was observed in TCP 224 (38.26) with 1.36 folds variation. TCP 154 showed highest hue angle (80.36) whereas lowest was found in TCP 2 (63.40) with 1.27 folds variation. The colour of the rhizomes of some of the studied genotypes has been given in Plate 4.1 which distinctly indicates wide variation in colour among the genotypes. It was observed from the results of colour estimation as well as from Plate 4.1 that rhizome of most of the genotypes under Group III with high curcumin level possessed deep

yellow to deep orange colour whereas those of Group II having medium range of curcumin demonstrated light yellow to deep yellow colour. The genotypes under Group I having low curcumin exhibited mainly light yellow colour.

Table 4.17: Results of color parameters in the studied genotypes

Group	Genotype	L* (mean* $\pm$ SE)	a* (mean* $\pm$ SE)	b* (mean* $\pm$ SE)	Hue angle (h°) (mean* $\pm$ SE)
Low	TCP 154	65.63 $\pm$ 0.159	8.69 $\pm$ 0.266	51.20 $\pm$ 0.390	80.36 $\pm$ 0.151
	TCP138	63.47 $\pm$ 0.039	9.70 $\pm$ 0.108	50.29 $\pm$ 0.424	79.09 $\pm$ 0.373
	TCP 7	62.37 $\pm$ 0.051	10.38 $\pm$ 0.224	50.99 $\pm$ 0.304	78.49 $\pm$ 0.091
	TCP 87	62.24 $\pm$ 0.135	10.30 $\pm$ 0.138	49.94 $\pm$ 0.164	78.35 $\pm$ 0.057
	TCP 100	64.40 $\pm$ 0.111	9.68 $\pm$ 0.196	52.19 $\pm$ 0.183	79.49 $\pm$ 0.165
	TCP 114	61.25 $\pm$ 0.064	10.39 $\pm$ 0.079	49.30 $\pm$ 0.402	78.09 $\pm$ 0.090
	TCP221	62.36 $\pm$ 0.135	10.51 $\pm$ 0.185	52.23 $\pm$ 0.249	78.62 $\pm$ 0.075
	TCP 63	63.66 $\pm$ 0.135	11.44 $\pm$ 0.183	48.94 $\pm$ 0.108	76.84 $\pm$ 0.499
	TCP 173	58.57 $\pm$ 0.096	10.54 $\pm$ 0.174	48.29 $\pm$ 0.041	77.69 $\pm$ 0.131
	TCP 152	59.62 $\pm$ 0.152	10.39 $\pm$ 0.178	49.51 $\pm$ 0.103	78.15 $\pm$ 0.086
	TCP 276	57.60 $\pm$ 0.146	9.71 $\pm$ 0.078	48.23 $\pm$ 0.086	78.62 $\pm$ 0.194
	TCP 198	60.17 $\pm$ 0.060	11.32 $\pm$ 0.068	52.12 $\pm$ 0.037	77.75 $\pm$ 0.169
	TCP 94	60.54 $\pm$ 0.157	11.17 $\pm$ 0.155	50.44 $\pm$ 0.052	77.52 $\pm$ 0.346
	TCP 225	58.18 $\pm$ 0.102	10.65 $\pm$ 0.080	51.35 $\pm$ 0.127	78.28 $\pm$ 0.037
	TCP 34	58.11 $\pm$ 0.106	10.87 $\pm$ 0.078	52.16 $\pm$ 0.139	78.22 $\pm$ 0.119
Medium	TCP 58	55.14 $\pm$ 0.174	10.71 $\pm$ 0.122	44.36 $\pm$ 0.116	76.43 $\pm$ 0.095
	TCP 93	50.21 $\pm$ 0.123	11.70 $\pm$ 0.076	41.37 $\pm$ 0.332	74.21 $\pm$ 0.092
	TCP 175	48.18 $\pm$ 0.106	15.57 $\pm$ 0.234	39.46 $\pm$ 0.211	68.47 $\pm$ 0.039
	TCP 224	46.21 $\pm$ 0.110	13.47 $\pm$ 0.131	38.26 $\pm$ 0.177	70.61 $\pm$ 0.082
	TCP 40	47.18 $\pm$ 0.112	14.72 $\pm$ 0.071	39.64 $\pm$ 0.134	69.63 $\pm$ 0.035
	TCP 139	47.37 $\pm$ 0.134	16.46 $\pm$ 0.116	41.39 $\pm$ 0.085	68.32 $\pm$ 0.086
	TCP 84	48.57 $\pm$ 0.165	18.38 $\pm$ 0.144	44.35 $\pm$ 0.166	67.49 $\pm$ 0.094
	TCP 137	46.87 $\pm$ 0.235	15.41 $\pm$ 0.085	40.52 $\pm$ 0.254	69.18 $\pm$ 0.289
	TCP 64	45.61 $\pm$ 0.203	16.37 $\pm$ 0.100	38.46 $\pm$ 0.139	66.94 $\pm$ 0.333
	TCP 202	48.78 $\pm$ 0.121	14.62 $\pm$ 0.071	38.41 $\pm$ 0.201	69.16 $\pm$ 0.174
	TCP 240	49.63 $\pm$ 0.173	14.48 $\pm$ 0.153	42.28 $\pm$ 0.226	71.09 $\pm$ 0.140
	TCP 12	47.82 $\pm$ 0.092	13.58 $\pm$ 0.203	41.34 $\pm$ 0.191	71.81 $\pm$ 0.106
	TCP 194	44.31 $\pm$ 0.078	15.36 $\pm$ 0.054	39.62 $\pm$ 0.234	68.81 $\pm$ 0.128
	TCP 222	46.71 $\pm$ 0.208	16.50 $\pm$ 0.076	40.57 $\pm$ 0.225	67.87 $\pm$ 0.031
	TCP 247	48.45 $\pm$ 0.186	15.43 $\pm$ 0.283	38.58 $\pm$ 0.208	68.20 $\pm$ 0.259
High	TCP 227	46.54 $\pm$ 0.188	14.59 $\pm$ 0.146	39.57 $\pm$ 0.255	69.76 $\pm$ 0.246
	TCP 50	48.54 $\pm$ 0.121	16.71 $\pm$ 0.082	44.35 $\pm$ 0.202	69.33 $\pm$ 0.083
	TCP 96	47.58 $\pm$ 0.163	16.66 $\pm$ 0.114	46.54 $\pm$ 0.191	70.31 $\pm$ 0.156
	TCP 57	49.67 $\pm$ 0.096	18.24 $\pm$ 0.057	47.61 $\pm$ 0.221	69.04 $\pm$ 0.188
	TCP 54	50.40 $\pm$ 0.271	17.34 $\pm$ 0.190	44.44 $\pm$ 0.160	68.68 $\pm$ 0.076
	TCP 181	49.56 $\pm$ 0.261	18.28 $\pm$ 0.093	43.35 $\pm$ 0.166	67.14 $\pm$ 0.179
	TCP 178	51.53 $\pm$ 0.216	21.27 $\pm$ 0.134	44.62 $\pm$ 0.194	64.51 $\pm$ 0.218
	TCP 11	50.41 $\pm$ 0.259	20.74 $\pm$ 0.112	44.60 $\pm$ 0.213	65.06 $\pm$ 0.425
	TCP 242	52.16 $\pm$ 0.116	21.38 $\pm$ 0.099	45.47 $\pm$ 0.263	64.82 $\pm$ 0.491
	TCP 196	51.43 $\pm$ 0.142	20.23 $\pm$ 0.088	43.46 $\pm$ 0.142	65.04 $\pm$ 0.229
	TCP 244	48.69 $\pm$ 0.213	21.54 $\pm$ 0.057	44.47 $\pm$ 0.137	64.16 $\pm$ 0.300
	TCP 200	49.59 $\pm$ 0.155	20.27 $\pm$ 0.171	43.59 $\pm$ 0.277	65.07 $\pm$ 0.513
	TCP 67	52.45 $\pm$ 0.257	22.10 $\pm$ 0.037	45.65 $\pm$ 0.248	64.17 $\pm$ 0.220
	TCP 195	51.26 $\pm$ 0.156	22.29 $\pm$ 0.162	44.59 $\pm$ 0.195	63.44 $\pm$ 0.090
	TCP 2	51.63 $\pm$ 0.245	22.73 $\pm$ 0.119	45.38 $\pm$ 0.086	63.40 $\pm$ 0.533

*Average of three replicates.

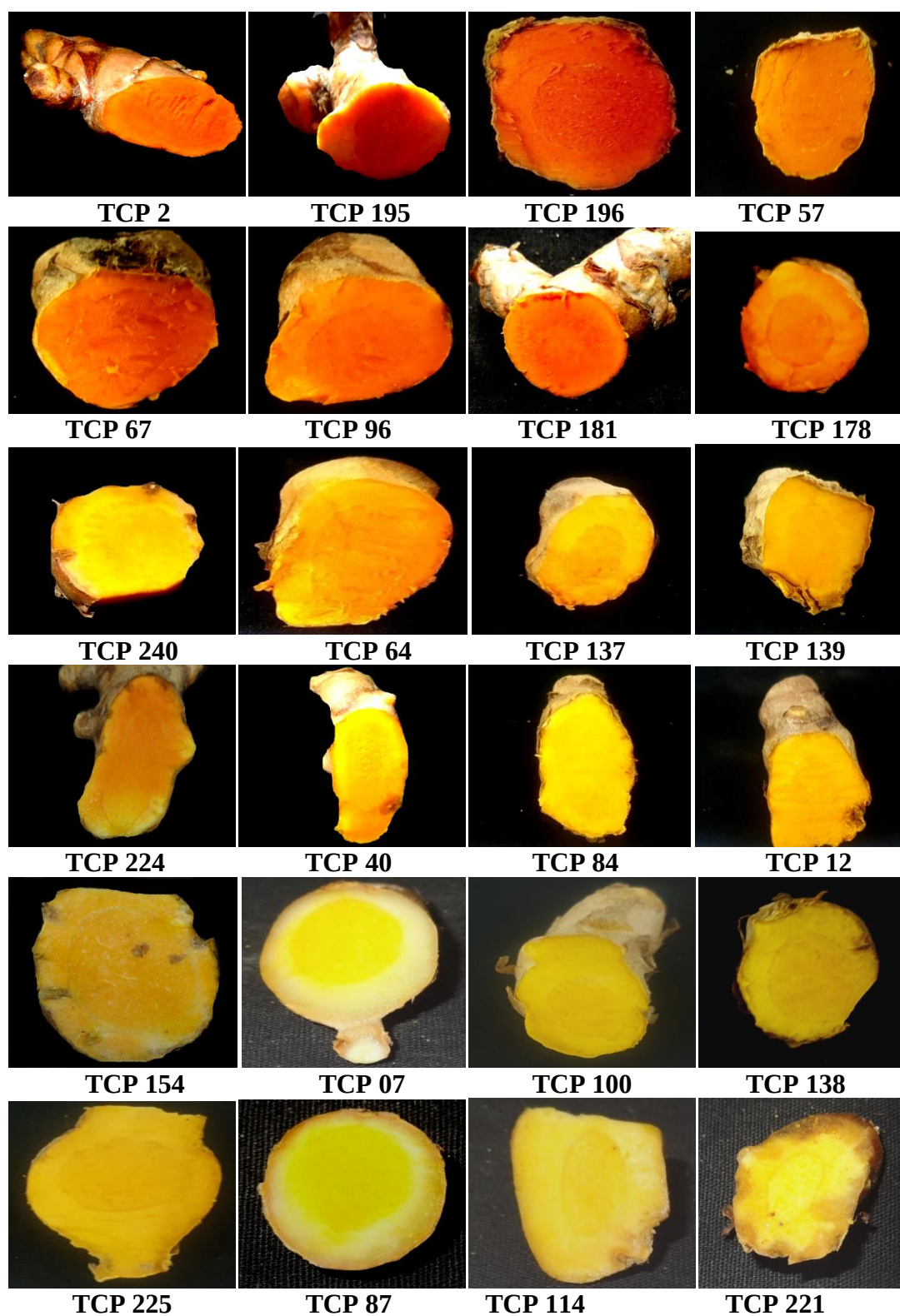


Plate 4.1: Variation in rhizome colour among some studied genotypes (first two rows from top are of Group III, next two of Group II and last two of Group I)

Madhusankha *et al.* (2018) did similar type of work in turmeric with five varieties and compared rhizome colour with curcumin content. They observed that visual colour difference ranged from bright yellow to orange yellow and concluded that this colour difference might be due to variation in curcumin content and the bright yellow colour came mainly from polyphenolic curcuminoid pigments. Houghton and Hendry (1992) have reported that ground turmeric powder is insoluble in water and imparts colour either by dispersion throughout the food or by dissolution of the curcumin into vegetable oil.

In the present study turmeric genotypes have been collected from the AICRP Centre at Pundibari with wide variation in rhizome colour. It has been found that variations in rhizome color of turmeric genotypes are associated with chemical composition, particularly iron content, antioxidant activity and pH homeostasis.

From the results it can be concluded that when curcumin content increases, colour of the rhizome tends towards deep orange colour. High curcumin containing genotypes also contained high a^* value and appeared towards deep orange colour whereas low curcumin containing genotypes showed high b^* value and imparted towards light yellow colour.

4.3.3 Total phenol, protein, iron and crude fat content

The principal bioactive component in turmeric rhizomes is curcumin, a member of phenolic group of biomolecules. In addition, there may be some other phenolic compounds which can play role in the antioxidant activities as well as defense mechanism of turmeric plants. It was, therefore, thought worthwhile to estimate total phenols present in rhizome powder of selected turmeric genotypes.

Turmeric is consumed as food additive and a substantial amount is used particularly in Indian curries and other dishes. So protein estimation was done to get an idea about its amount that can be obtained from turmeric rhizomes and also to make a comparative study between their protein content and antioxidant enzyme activity. Similarly content of iron, an essential mineral for human body, was also estimated to assess its availability and also to find its relation with the colour of the turmeric rhizomes.

Crude fat content in the studied turmeric genotypes was also estimated as it is an important constituent of turmeric being the complex mixture of secondary plant metabolites responsible for its diverse bioactivity including medicinal properties.

From the results (Table 4.18) of total phenol content it was evident that majority of the turmeric genotypes under Group II and III contained high total phenol (TCP 222, TCP 84, TCP 64, TCP 194, TCP 2, TCP 67 and TCP 195) whereas those of Group I possessed low total phenol. Highest phenol was present in TCP 247 (9.96 mg GAE/g) of Group II and lowest in TCP 138 (2.86 mg GAE/g), a member of Group I making 3.48 folds variation with respect to the highest one.

Curcuminoids being phenolic in nature contribute significantly to the total phenolics level of turmeric rhizomes. The reason of exhibiting high phenol content by turmeric with high curcumin has been explained by Borra *et al.* (2013) as the two phenolic hydroxyl groups of curcumin being linked to different nonadjacent benzene rings make them work freely without hindrance.

It was observed from the results (Table 4.18) that there was a parallel trend between curcumin and protein content among the turmeric genotypes. The genotypes belonging to Group III contained high protein level (TCP 227, TCP 195 and TCP 2), Group II possessed medium protein containing (TCP 58, TCP 175 and TCP 222) whereas Group I contained mainly low protein containing genotypes. Here, total protein content was found highest in TCP 227 (42.42 mg/g) of Group III whereas lowest was found in TCP 100 (15.38 mg/g) Group I with 2.76 folds variation in respect to the highest one.

The occurrence of high curcumin and high protein in the same plant might be due to the presence of higher amount of enzymes involved in curcumin synthesis like curcumin synthase, diketide-CoA synthase etc. in the phenylpropanoid pathway of curcumin biosynthesis (Katsuyama *et al.*, 2009). However, further investigation needs for its confirmation.

The results revealed that majority of the genotypes under Group II and III (notably TCP 240, TCP 12, TCP 194, TCP 227, TCP 181, TCP 2 and TCP 195) possessed high iron whereas Group I contained mainly low iron containing genotypes. In the present study, highest iron was present in TCP 195 (55.09 mg/100g) whereas lowest was in TCP 221 (46.79 mg/100g) with 1.18 folds variation

with regards to the highest one. So it reveals that iron content is also of similar trend with the curcumin content in turmeric rhizomes.

Table 4.18: Total phenol, protein, iron and crude fat contents in the studied genotypes of *Curcuma longa*

Group	Genotype	Total phenol (mg GAE/g dry rhizome) (mean* $\pm$ SE)	Protein content (mg/ g) (mean* $\pm$ SE)	Iron content (mg/100 g) (mean* $\pm$ SE)	Crude fat content (%) (mean* $\pm$ SE)
Low	TCP 154	3.29 $\pm$ 0.026	20.42 $\pm$ 0.159	47.15 $\pm$ 0.524	5.13 $\pm$ 0.025
	TCP138	2.86 $\pm$ 0.023	23.54 $\pm$ 0.668	48.31 $\pm$ 0.294	4.49 $\pm$ 0.096
	TCP 7	3.06 $\pm$ 0.029	21.20 $\pm$ 0.233	47.92 $\pm$ 1.106	3.55 $\pm$ 0.025
	TCP 87	3.46 $\pm$ 0.053	25.80 $\pm$ 0.425	48.23 $\pm$ 0.315	5.23 $\pm$ 0.029
	TCP 100	4.31 $\pm$ 0.053	15.38 $\pm$ 0.181	47.54 $\pm$ 0.290	6.94 $\pm$ 0.019
	TCP 114	2.89 $\pm$ 0.056	20.52 $\pm$ 0.208	50.58 $\pm$ 0.407	2.42 $\pm$ 0.011
	TCP221	4.37 $\pm$ 0.071	21.08 $\pm$ 0.313	46.79 $\pm$ 0.382	6.84 $\pm$ 0.014
	TCP 63	2.88 $\pm$ 0.046	24.24 $\pm$ 0.227	51.06 $\pm$ 0.324	2.33 $\pm$ 0.015
	TCP 173	4.61 $\pm$ 0.126	20.04 $\pm$ 0.085	50.08 $\pm$ 0.198	2.63 $\pm$ 0.011
	TCP 152	2.57 $\pm$ 0.084	24.51 $\pm$ 0.234	50.02 $\pm$ 0.192	1.32 $\pm$ 0.022
	TCP 276	4.52 $\pm$ 0.075	36.39 $\pm$ 0.172	50.65 $\pm$ 0.268	2.43 $\pm$ 0.007
	TCP 198	4.06 $\pm$ 0.093	20.37 $\pm$ 0.128	52.56 $\pm$ 0.682	2.31 $\pm$ 0.014
	TCP 94	4.24 $\pm$ 0.072	27.50 $\pm$ 0.209	50.26 $\pm$ 0.495	2.41 $\pm$ 0.014
	TCP 225	5.09 $\pm$ 0.039	16.61 $\pm$ 0.170	49.93 $\pm$ 0.333	1.45 $\pm$ 0.015
	TCP 34	5.07 $\pm$ 0.029	28.47 $\pm$ 0.276	51.69 $\pm$ 0.283	1.34 $\pm$ 0.011
Medium	TCP 58	3.60 $\pm$ 0.052	30.40 $\pm$ 0.152	50.87 $\pm$ 0.198	2.54 $\pm$ 0.011
	TCP 93	3.97 $\pm$ 0.043	23.35 $\pm$ 0.125	49.72 $\pm$ 0.125	1.94 $\pm$ 0.011
	TCP 175	4.36 $\pm$ 0.070	32.73 $\pm$ 0.349	49.02 $\pm$ 0.426	5.63 $\pm$ 0.007
	TCP 224	7.46 $\pm$ 0.121	23.97 $\pm$ 0.184	48.71 $\pm$ 0.301	3.65 $\pm$ 0.011
	TCP 40	8.48 $\pm$ 0.106	24.35 $\pm$ 0.143	51.44 $\pm$ 0.194	2.70 $\pm$ 0.018
	TCP 139	7.15 $\pm$ 0.088	24.29 $\pm$ 0.195	48.78 $\pm$ 0.355	3.76 $\pm$ 0.007
	TCP 84	9.74 $\pm$ 0.078	29.52 $\pm$ 0.226	49.41 $\pm$ 0.329	4.26 $\pm$ 0.011
	TCP 137	8.94 $\pm$ 0.073	28.35 $\pm$ 0.195	51.73 $\pm$ 0.246	3.71 $\pm$ 0.011
	TCP 64	9.65 $\pm$ 0.068	26.73 $\pm$ 0.031	50.73 $\pm$ 0.355	3.31 $\pm$ 0.011
	TCP 202	5.37 $\pm$ 0.099	24.25 $\pm$ 0.073	48.62 $\pm$ 0.991	4.66 $\pm$ 0.011
	TCP 240	7.72 $\pm$ 0.085	27.31 $\pm$ 0.073	54.18 $\pm$ 0.191	3.56 $\pm$ 0.008
	TCP 12	8.27 $\pm$ 0.078	28.54 $\pm$ 0.262	55.02 $\pm$ 0.195	4.06 $\pm$ 0.007
	TCP 194	9.62 $\pm$ 0.097	27.52 $\pm$ 0.122	54.77 $\pm$ 0.289	1.89 $\pm$ 0.014
	TCP 222	9.85 $\pm$ 0.071	32.58 $\pm$ 0.252	52.18 $\pm$ 0.386	2.33 $\pm$ 0.007
	TCP 247	9.96 $\pm$ 0.054	22.54 $\pm$ 0.157	53.09 $\pm$ 0.222	4.06 $\pm$ 0.018
High	TCP 227	8.53 $\pm$ 0.075	42.42 $\pm$ 0.307	54.90 $\pm$ 0.271	2.16 $\pm$ 0.011
	TCP 50	6.82 $\pm$ 0.045	33.11 $\pm$ 0.334	52.17 $\pm$ 0.184	3.37 $\pm$ 0.007
	TCP 96	8.91 $\pm$ 0.112	27.14 $\pm$ 0.029	53.66 $\pm$ 0.980	2.76 $\pm$ 0.011
	TCP 57	8.81 $\pm$ 0.063	27.61 $\pm$ 0.096	52.80 $\pm$ 0.943	2.45 $\pm$ 0.007
	TCP 54	5.36 $\pm$ 0.064	27.33 $\pm$ 0.096	50.67 $\pm$ 0.584	2.03 $\pm$ 0.007
	TCP 181	7.66 $\pm$ 0.068	31.68 $\pm$ 0.261	54.24 $\pm$ 0.306	2.15 $\pm$ 0.007
	TCP 178	7.61 $\pm$ 0.086	23.48 $\pm$ 0.115	53.74 $\pm$ 0.315	2.32 $\pm$ 0.007
	TCP 11	5.77 $\pm$ 0.074	33.67 $\pm$ 0.044	53.10 $\pm$ 0.333	4.82 $\pm$ 0.011
	TCP 242	7.95 $\pm$ 0.058	25.42 $\pm$ 0.164	50.46 $\pm$ 0.238	2.76 $\pm$ 0.011
	TCP 196	7.18 $\pm$ 0.067	25.82 $\pm$ 0.115	51.77 $\pm$ 0.237	2.53 $\pm$ 0.007
	TCP 244	5.56 $\pm$ 0.072	23.82 $\pm$ 0.092	52.31 $\pm$ 0.302	2.43 $\pm$ 0.011
	TCP 200	6.58 $\pm$ 0.068	35.48 $\pm$ 0.196	51.62 $\pm$ 0.415	3.23 $\pm$ 0.011
	TCP 67	9.32 $\pm$ 0.073	37.50 $\pm$ 0.116	51.76 $\pm$ 0.265	3.26 $\pm$ 0.011
	TCP 195	9.48 $\pm$ 0.087	39.56 $\pm$ 0.186	55.09 $\pm$ 0.103	2.13 $\pm$ 0.011
	TCP 2	9.00 $\pm$ 0.048	41.37 $\pm$ 0.086	53.34 $\pm$ 0.190	3.56 $\pm$ 0.007

*Average of three replicates.

In case of crude fat the trend of the result (Table 4.18) was a little different with regards to curcumin content. The group with low curcumin possessed higher amounts of crude fats, *e.g.* TCP 100, TCP 221, TCP 87 and TCP 154 of Group I whereas TCP 175, TCP 84, TCP 202, TCP 12 and TCP 247 of Group II possessed medium level and majority of Group III contained low crude fat containing genotypes. In the present study, TCP 100 showed the highest (6.94%) crude fat content whereas TCP 152 contained the lowest (1.32%) percent with 5.26 folds variation with the highest one.

The content of crude fat in the studied rhizomes were similar to earlier studies of Fattepurkar *et al.* (2009) who found 2.66 to 4.46% crude fat in the turmeric finger rhizomes and that of Mane *et al.* (2018) who observed 1.08% fat in fresh turmeric rhizomes. In another study, Ahaotu and Lawal (2019) found 0.80% fat in *C. longa* rhizomes.

4.3.4 Assay of antioxidant activity

Antioxidants, substances that inhibit the oxidation of other compounds, include enzymes, vitamins, thiols, polyphenols and other compounds. Turmeric is a good source of antioxidant compounds. Assay of antioxidant activity of the turmeric varieties was done by four different methods (DPPH, ABTS, TAC and CUPRAC) in the present study. The results of DPPH and ABTS activity have been expressed in terms of IC₅₀ value.

The IC₅₀ value is inversely related to the antioxidant capacity of a compound, because it implies the amount of antioxidant required to reduce the concentration of free radical to 50% of its initial value. Lower the IC₅₀ value, higher is the antioxidant activity of a compound (Li *et al.*, 2009).

So from the result as recorded in Table 4.19 it was found that in case of DPPH and ABTS free radical scavenging activity, Group III contained genotypes with high activity, Group II possessed medium activity containing genotypes whereas Group I had low activity containing genotypes. TCP 195 of Group III exhibited maximum (78.17 µg/ml) DPPH free radical scavenging activity followed by TCP 242 (84.52 µg/ml), TCP 196 (88.66 µg/ml), TCP 57 (89.87 µg/ml), TCP 54

(94.39 $\mu\text{g/ml}$) and TCP 2 (95.50 $\mu\text{g/ml}$) whereas lowest value (294.79 $\mu\text{g/ml}$) was found in TCP 114 with 3.77 folds variation with respect to the highest one.

Table 4.19: Antioxidant assay of the studied genotypes of *Curcuma longa*

Group	Genotype	A [#] (mean* $\pm$ SE) IC ₅₀ ($\mu\text{g/ml}$)	B [#] (mean* $\pm$ SE) IC ₅₀ ($\mu\text{g/ml}$)	C [#] (mean* $\pm$ SE)	D [#] (mean* $\pm$ SE)
Low	TCP 154	280.31 $\pm$ 0.716	275.28 $\pm$ 2.700	13.43 $\pm$ 0.170	0.74 $\pm$ 0.033
	TCP138	285.68 $\pm$ 0.835	273.13 $\pm$ 3.675	10.80 $\pm$ 0.078	0.52 $\pm$ 0.022
	TCP 7	272.74 $\pm$ 1.212	253.72 $\pm$ 0.999	12.66 $\pm$ 0.119	0.65 $\pm$ 0.014
	TCP 87	268.10 $\pm$ 1.166	283.85 $\pm$ 1.110	14.49 $\pm$ 0.304	0.75 $\pm$ 0.022
	TCP 100	275.15 $\pm$ 1.832	289.63 $\pm$ 1.425	13.62 $\pm$ 0.241	0.91 $\pm$ 0.029
	TCP 114	294.79 $\pm$ 1.513	286.40 $\pm$ 1.259	21.43 $\pm$ 0.246	0.81 $\pm$ 0.021
	TCP221	288.76 $\pm$ 1.744	297.96 $\pm$ 1.007	21.51 $\pm$ 0.140	0.95 $\pm$ 0.025
	TCP 63	258.54 $\pm$ 2.020	277.58 $\pm$ 1.427	13.43 $\pm$ 0.259	0.68 $\pm$ 0.029
	TCP 173	267.02 $\pm$ 1.069	279.87 $\pm$ 0.972	9.46 $\pm$ 0.113	0.76 $\pm$ 0.029
	TCP 152	244.08 $\pm$ 1.102	226.55 $\pm$ 1.524	19.41 $\pm$ 0.297	0.71 $\pm$ 0.025
	TCP 276	226.46 $\pm$ 1.514	209.14 $\pm$ 1.826	13.80 $\pm$ 0.184	0.76 $\pm$ 0.032
	TCP 198	238.38 $\pm$ 1.125	245.87 $\pm$ 1.164	20.55 $\pm$ 0.243	0.72 $\pm$ 0.025
	TCP 94	193.87 $\pm$ 1.169	217.22 $\pm$ 1.814	18.37 $\pm$ 0.226	0.81 $\pm$ 0.015
	TCP 225	216.50 $\pm$ 1.453	229.84 $\pm$ 1.104	10.57 $\pm$ 0.236	0.94 $\pm$ 0.029
Medium	TCP 34	197.54 $\pm$ 0.809	216.49 $\pm$ 1.553	11.73 $\pm$ 0.195	0.84 $\pm$ 0.015
	TCP 58	173.96 $\pm$ 1.088	187.91 $\pm$ 1.068	22.55 $\pm$ 0.373	0.73 $\pm$ 0.032
	TCP 93	179.42 $\pm$ 1.389	195.45 $\pm$ 1.238	20.09 $\pm$ 0.305	1.41 $\pm$ 0.025
	TCP 175	187.28 $\pm$ 1.079	179.83 $\pm$ 0.938	22.51 $\pm$ 0.361	2.17 $\pm$ 0.032
	TCP 224	158.18 $\pm$ 1.040	177.61 $\pm$ 1.508	17.53 $\pm$ 0.238	2.28 $\pm$ 0.049
	TCP 40	163.96 $\pm$ 1.068	153.50 $\pm$ 1.561	28.17 $\pm$ 0.460	2.95 $\pm$ 0.022
	TCP 139	162.30 $\pm$ 0.679	144.33 $\pm$ 1.313	33.95 $\pm$ 0.315	2.69 $\pm$ 0.018
	TCP 84	144.60 $\pm$ 1.577	137.52 $\pm$ 1.432	32.03 $\pm$ 0.521	2.67 $\pm$ 0.039
	TCP 137	164.99 $\pm$ 1.117	158.21 $\pm$ 1.228	29.43 $\pm$ 0.236	2.83 $\pm$ 0.018
	TCP 64	146.65 $\pm$ 3.931	127.54 $\pm$ 1.255	20.64 $\pm$ 0.331	2.05 $\pm$ 0.028
	TCP 202	151.10 $\pm$ 1.655	126.80 $\pm$ 1.255	37.18 $\pm$ 0.470	3.19 $\pm$ 0.018
	TCP 240	146.63 $\pm$ 1.109	131.11 $\pm$ 1.113	31.37 $\pm$ 0.294	2.32 $\pm$ 0.029
	TCP 12	133.50 $\pm$ 1.406	120.02 $\pm$ 0.919	33.99 $\pm$ 0.563	2.84 $\pm$ 0.018
	TCP 194	145.96 $\pm$ 1.091	123.67 $\pm$ 0.958	19.79 $\pm$ 0.414	1.92 $\pm$ 0.027
High	TCP 222	128.16 $\pm$ 1.013	116.49 $\pm$ 1.032	29.91 $\pm$ 0.187	2.83 $\pm$ 0.029
	TCP 247	127.57 $\pm$ 1.405	116.78 $\pm$ 1.294	33.95 $\pm$ 0.176	2.39 $\pm$ 0.031
	TCP 227	122.12 $\pm$ 1.675	132.83 $\pm$ 1.255	27.71 $\pm$ 0.227	2.60 $\pm$ 0.046
	TCP 50	117.66 $\pm$ 1.439	128.78 $\pm$ 0.965	19.50 $\pm$ 0.354	2.61 $\pm$ 0.040
	TCP 96	102.26 $\pm$ 1.302	115.06 $\pm$ 1.012	27.79 $\pm$ 0.281	2.22 $\pm$ 0.029
	TCP 57	89.87 $\pm$ 1.030	99.30 $\pm$ 0.937	22.75 $\pm$ 0.222	2.73 $\pm$ 0.019
	TCP 54	94.39 $\pm$ 1.344	114.57 $\pm$ 0.655	26.24 $\pm$ 0.400	3.05 $\pm$ 0.042
	TCP 181	115.20 $\pm$ 1.360	105.13 $\pm$ 1.265	41.61 $\pm$ 0.549	2.77 $\pm$ 0.040
	TCP 178	107.09 $\pm$ 1.920	118.69 $\pm$ 0.768	31.23 $\pm$ 0.206	2.90 $\pm$ 0.029
	TCP 11	97.78 $\pm$ 1.016	109.62 $\pm$ 1.133	40.86 $\pm$ 1.149	3.08 $\pm$ 0.032
	TCP 242	84.52 $\pm$ 0.575	106.82 $\pm$ 1.257	30.46 $\pm$ 0.760	2.92 $\pm$ 0.039
	TCP 196	88.66 $\pm$ 1.097	104.17 $\pm$ 1.020	32.33 $\pm$ 0.688	3.72 $\pm$ 0.025
	TCP 244	97.90 $\pm$ 1.175	109.48 $\pm$ 0.764	39.78 $\pm$ 0.093	3.53 $\pm$ 0.042
	TCP 200	124.81 $\pm$ 2.157	101.95 $\pm$ 1.105	42.40 $\pm$ 0.731	3.92 $\pm$ 0.029
	TCP 67	113.30 $\pm$ 1.482	97.90 $\pm$ 1.271	53.76 $\pm$ 0.398	3.83 $\pm$ 0.036
	TCP 195	78.17 $\pm$ 1.091	89.26 $\pm$ 0.641	57.52 $\pm$ 0.544	4.42 $\pm$ 0.031
	TCP 2	95.50 $\pm$ 0.652	107.72 $\pm$ 1.227	55.63 $\pm$ 0.490	4.17 $\pm$ 0.025

*Average of three replicates ($\mu\text{g/ml}$)

[#] [A: DPPH free radical scavenging activity, B: ABTS free radical scavenging activity, C: Total antioxidant activity (mg/g AAE), D: CUPRAC assay (mmol TE/g)]

ABTS free radical scavenging activity was also found maximum in TCP 195 (89.26 µg/ml) followed by TCP 67 (97.90 µg/ml), TCP 57 (99.30 µg/ml), TCP 200 (101.95 µg/ml), TCP 196 (104.17 µg/ml), TCP 242 (106.82 µg/ml) and TCP 2 (107.72 µg/ml) whereas lowest was recorded in TCP 221 (297.96 µg/ml) with 3.34 folds variation.

In case of TAC, TCP 195 contained maximum activity (57.52 mg/g AAE) followed by TCP 2 (55.63 mg/g AAE), TCP 67 (53.76 mg/g AAE), TCP 200 (42.40 mg/g AAE) and TCP 181 (41.61 mg/g AAE) whereas lowest was found in TCP 173 (9.46 mg/g AAE) with 6.08 folds variation.

CUPRAC assay also showed maximum activity in TCP 195 (4.42 mmol TE/g) followed by TCP 2 (4.17 mmol TE/g), TCP 200 (3.92 mmol TE/g), TCP 67 (3.83 mmol TE/g) and TCP 196 (3.72 mmol TE/g) whereas lowest was recorded in TCP 138 (0.52 mmol TE/g) with 8.50 folds variation in respect to the highest one.

Scavenging activity might be attributed to high reducing power and higher total phenolic contents present in curcumin. Curcumin is a unique antioxidant, which contains a variety of functional groups (Jovanovic *et al.*, 1999). It reacts as a scavenger of oxygen free radicals (Subramanian *et al.*, 1994). It can also inhibit the generation of reactive oxygen species (ROS) like super oxide anions, H₂O₂ and nitrite radical generation.

The antioxidant mechanism of curcumin is assigned to its unique conjugated structure which includes two methoxylated phenols and end form of β-diketone. The structure exhibits radical trapping ability as a chain breaking antioxidant. Turmeric with active constituent curcumin showed high antioxidant activity to inhibit lipid peroxidation & cyclooxygenase activity (Hussain, 2002). The antioxidant activity of turmeric increases when the phenolic group with a methoxy group is at the *ortho*-position (Motterlini *et al.*, 2000).

The experimental findings exhibit that turmeric contains considerable amounts of phenols due to which their free radical scavenging power is comparable with gallic acid. The need to use various methods of antioxidant capacity measurement is due to the different mechanisms of antioxidant action. Determination of the antioxidant activity of plant extracts and compounds often show different results as the methods used are based on different reaction mechanisms (Wong *et al.*,

2006). Wojdylo *et al.* (2007) studied the total antioxidant capacity of 32 turmeric species analyzing by ABTS, DPPH and FRAP method and found the following results: 19.5 μM Trolox/100 g (ABTS), 100 μM Trolox/100 g (DPPH) and 62.6 μM Trolox/100 g (FRAP) respectively.

4.3.5 Antioxidant enzyme assay

The antioxidant activity of curcumin could be moderated through antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase. These antioxidant enzymes are the main line of defense against free radicals in animal and plant cells. The primary antioxidant enzymes are superoxide dismutase (SOD), catalase and peroxidases. It is interesting to note that all three selected antioxidant enzyme uses iron as their cofactor. Three different methods were used to estimate the antioxidant enzyme activity of fresh rhizomes of turmeric.

From the result it was found that turmeric varieties of Group III exhibited highest antioxidant enzyme activity, Group II possessed medium activity while Group I had low antioxidant enzyme activity.

In the present study, catalase activity was found highest in TCP 67 (11.22 $\Delta\text{OD}/\text{min}/\text{g}$ of fresh wt) followed by TCP 2 (10.97 $\Delta\text{OD}/\text{min}/\text{g}$ of fresh wt), TCP 195 (10.92 $\Delta\text{OD}/\text{min}/\text{g}$ of fresh wt), TCP 200 (9.59 $\Delta\text{OD}/\text{min}/\text{g}$ of fresh wt) and TCP 244 (9.22 $\Delta\text{OD}/\text{min}/\text{g}$ of fresh wt) while lowest was recorded in TCP 154 (2.16 $\Delta\text{OD}/\text{min}/\text{g}$ of fresh wt) with 5.19 folds variation with respect to the highest one (Table 4.20).

TCP 195 showed highest peroxidase activity (8.21 $\Delta\text{OD}/\text{min}/\text{g}$ of fresh wt) followed by TCP 2 (7.93 $\Delta\text{OD}/\text{min}/\text{g}$ of fresh wt), TCP 244 (7.71 $\Delta\text{OD}/\text{min}/\text{g}$ of fresh wt), TCP 200 (7.62 $\Delta\text{OD}/\text{min}/\text{g}$ of fresh wt) and TCP 67 (7.44 $\Delta\text{OD}/\text{min}/\text{g}$ of fresh wt) whereas lowest was recorded in TCP 87 (1.46 $\Delta\text{OD}/\text{min}/\text{g}$ of fresh wt) with 5.62 folds variation.

The SOD activity was found highest in TCP 244 (3.05 units/mg protein) followed by TCP 196 (2.56 units/mg protein), TCP 242 (2.34 units/mg protein), TCP 178 (2.23 units/mg protein) and TCP 195 (2.14 units/mg protein) while lowest was recorded in TCP 276 (0.43 units/mg protein) with 7.09 folds variation with respect to the highest one.

Table 4.20: Antioxidant enzyme assay of the studied genotypes of *Curcuma longa*

Group	Genotype	Catalase (Δ OD/min/g of fresh wt) (mean* $\pm$ SE)	Peroxidase (Δ OD/min/g of fresh wt) (mean* $\pm$ SE)	SOD (units/mg protein) (mean* $\pm$ SE)
Low	TCP 154	2.16 $\pm$ 0.044	1.83 $\pm$ 0.065	0.64 $\pm$ 0.011
	TCP138	2.44 $\pm$ 0.044	1.75 $\pm$ 0.062	0.57 $\pm$ 0.007
	TCP 7	2.76 $\pm$ 0.037	1.96 $\pm$ 0.140	0.64 $\pm$ 0.007
	TCP 87	3.56 $\pm$ 0.037	1.46 $\pm$ 0.060	0.51 $\pm$ 0.018
	TCP 100	2.85 $\pm$ 0.037	2.24 $\pm$ 0.086	0.86 $\pm$ 0.011
	TCP 114	3.39 $\pm$ 0.049	2.15 $\pm$ 0.138	0.65 $\pm$ 0.011
	TCP221	3.31 $\pm$ 0.049	2.26 $\pm$ 0.079	0.63 $\pm$ 0.011
	TCP 63	3.91 $\pm$ 0.037	2.73 $\pm$ 0.057	0.54 $\pm$ 0.011
	TCP 173	4.21 $\pm$ 0.044	2.35 $\pm$ 0.025	0.66 $\pm$ 0.011
	TCP 152	4.11 $\pm$ 0.044	2.03 $\pm$ 0.079	0.54 $\pm$ 0.011
	TCP 276	4.93 $\pm$ 0.037	2.16 $\pm$ 0.025	0.43 $\pm$ 0.011
	TCP 198	5.24 $\pm$ 0.044	2.82 $\pm$ 0.043	0.76 $\pm$ 0.011
	TCP 94	5.06 $\pm$ 0.044	3.23 $\pm$ 0.037	0.71 $\pm$ 0.011
	TCP 225	5.64 $\pm$ 0.044	3.36 $\pm$ 0.067	1.17 $\pm$ 0.011
Medium	TCP 34	5.42 $\pm$ 0.032	2.96 $\pm$ 0.071	0.71 $\pm$ 0.011
	TCP 58	6.12 $\pm$ 0.032	4.05 $\pm$ 0.057	0.87 $\pm$ 0.015
	TCP 93	6.04 $\pm$ 0.051	4.47 $\pm$ 0.108	1.14 $\pm$ 0.015
	TCP 175	6.27 $\pm$ 0.037	4.53 $\pm$ 0.071	0.83 $\pm$ 0.011
	TCP 224	6.36 $\pm$ 0.037	5.30 $\pm$ 0.089	1.13 $\pm$ 0.011
	TCP 40	6.26 $\pm$ 0.063	5.23 $\pm$ 0.039	1.11 $\pm$ 0.015
	TCP 139	6.18 $\pm$ 0.044	4.61 $\pm$ 0.058	1.18 $\pm$ 0.011
	TCP 84	6.83 $\pm$ 0.032	4.86 $\pm$ 0.074	0.97 $\pm$ 0.011
	TCP 137	7.04 $\pm$ 0.044	5.22 $\pm$ 0.039	1.17 $\pm$ 0.011
	TCP 64	6.92 $\pm$ 0.039	5.43 $\pm$ 0.178	1.25 $\pm$ 0.015
	TCP 202	6.78 $\pm$ 0.044	5.90 $\pm$ 0.099	1.37 $\pm$ 0.011
	TCP 240	7.22 $\pm$ 0.053	5.44 $\pm$ 0.031	1.25 $\pm$ 0.015
	TCP 12	7.17 $\pm$ 0.037	5.46 $\pm$ 0.055	1.18 $\pm$ 0.011
	TCP 194	7.53 $\pm$ 0.032	6.55 $\pm$ 0.079	1.46 $\pm$ 0.015
High	TCP 222	7.38 $\pm$ 0.032	6.35 $\pm$ 0.108	1.22 $\pm$ 0.022
	TCP 247	7.33 $\pm$ 0.058	6.50 $\pm$ 0.097	1.76 $\pm$ 0.025
	TCP 227	7.81 $\pm$ 0.044	6.38 $\pm$ 0.197	0.95 $\pm$ 0.015
	TCP 50	7.68 $\pm$ 0.032	6.23 $\pm$ 0.121	1.37 $\pm$ 0.007
	TCP 96	7.92 $\pm$ 0.046	7.51 $\pm$ 0.156	1.71 $\pm$ 0.014
	TCP 57	7.86 $\pm$ 0.037	7.26 $\pm$ 0.092	1.67 $\pm$ 0.015
	TCP 54	8.22 $\pm$ 0.049	7.39 $\pm$ 0.087	1.68 $\pm$ 0.007
	TCP 181	8.09 $\pm$ 0.056	7.25 $\pm$ 0.025	1.67 $\pm$ 0.011
	TCP 178	8.08 $\pm$ 0.037	6.96 $\pm$ 0.049	2.23 $\pm$ 0.011
	TCP 11	8.08 $\pm$ 0.025	7.64 $\pm$ 0.138	1.77 $\pm$ 0.014
	TCP 242	8.12 $\pm$ 0.032	7.28 $\pm$ 0.097	2.34 $\pm$ 0.011
	TCP 196	8.53 $\pm$ 0.044	6.47 $\pm$ 0.045	2.56 $\pm$ 0.011
	TCP 244	9.22 $\pm$ 0.032	7.71 $\pm$ 0.053	3.05 $\pm$ 0.015
	TCP 200	9.59 $\pm$ 0.044	7.62 $\pm$ 0.022	2.05 $\pm$ 0.011
	TCP 67	11.22 $\pm$ 0.049	7.44 $\pm$ 0.148	2.11 $\pm$ 0.015
	TCP 195	10.92 $\pm$ 0.039	8.21 $\pm$ 0.129	2.14 $\pm$ 0.011
	TCP 2	10.97 $\pm$ 0.051	7.93 $\pm$ 0.071	2.13 $\pm$ 0.011

*Average of three replicates

Catalase is an antioxidant enzyme that plays an important role in the oxidative damage of cell by conversion of hydrogen peroxide to water and oxygen whereas peroxidase catalyzes the oxidation of a wide variety of organic and inorganic substrates by hydrogen peroxide. SOD is the most indispensable enzyme for protecting cells from the toxicity of the reactive oxygen species and catalyzes the simultaneous oxidation and reduction of the superoxide radical to produce oxygen and hydrogen peroxide (Harrison, 2013). Curcumin has been shown to act as a Michael acceptor, reacting with glutathione and thioredoxin (Adams *et al.*, 2005).

4.3.6 pH measurements

The acidic or basic nature of a solution is essential to determine the appropriate use of that solution. Generally, a weak acidic pH is most often maintained in turmeric plant cells and the slightest modification often lead to changes in curcumin stability, and consequently, rhizome colour. Turmeric is orange red or reddish brown as a natural dye, but it changes colour in the presence of acids or bases. Curcumin is comparatively stable at acidic pH but it rapidly decomposes at pH above neutral.

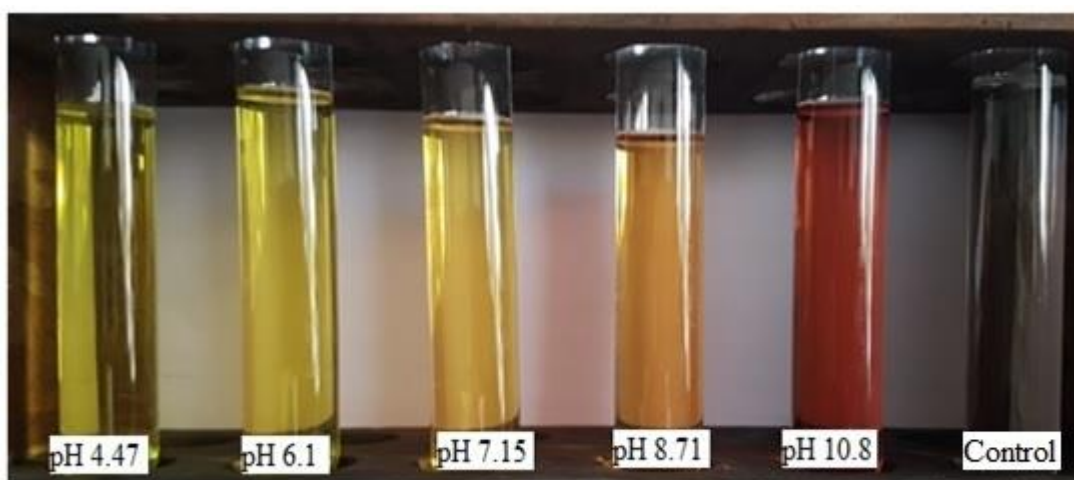


Plate 4.2: Colour differences occur at different pH when reaction occurs between curcumin and iron salt

A study on colour change in curcumin solution with change of pH as presented in Plate 4.2 showed that changing of pH from acidic to alkaline, colour turned from light yellow to dark brick red. It is, therefore, not acceptable as a coloring agent in aqueous solutions of pH greater than 7.00.

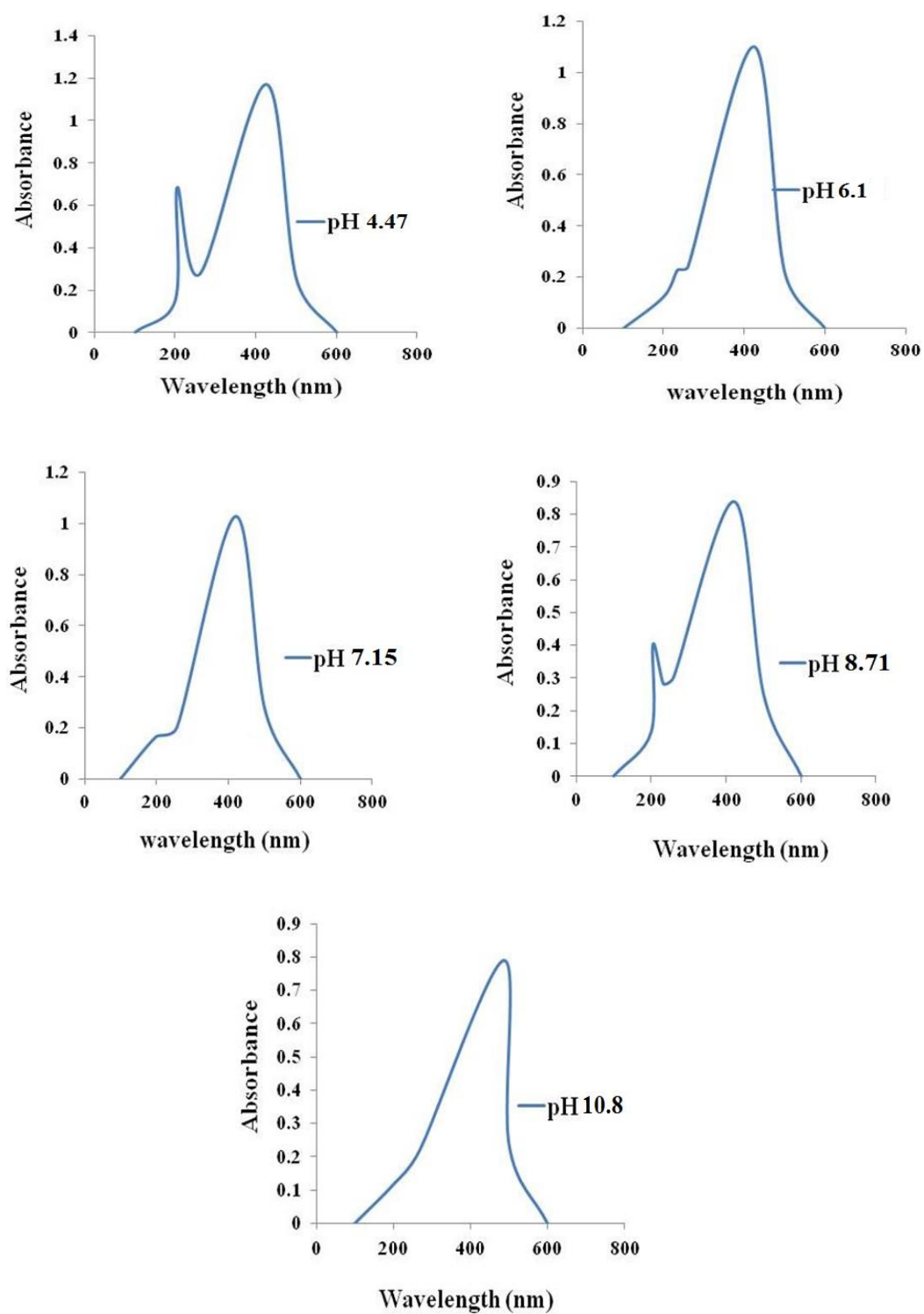


Fig. 4.5: Degradation of standard curcumin with change of pH

Table 4.21: pH measurements of the studied genotypes of *Curcuma longa*

Group	Genotype	Initial pH (mean* $\pm$ SEM)	Final pH (mean* $\pm$ SEM)	pH Difference (mean* $\pm$ SEM)
Low	TCP 154	7.54 $\pm$ 0.014	7.42 $\pm$ 0.007	0.12 $\pm$ 0.007
	TCP138	6.76 $\pm$ 0.011	6.67 $\pm$ 0.011	0.09 $\pm$ 0.011
	TCP 7	7.17 $\pm$ 0.011	7.01 $\pm$ 0.011	0.16 $\pm$ 0.011
	TCP 87	7.51 $\pm$ 0.011	7.24 $\pm$ 0.007	0.27 $\pm$ 0.007
	TCP 100	6.75 $\pm$ 0.021	6.70 $\pm$ 0.015	0.05 $\pm$ 0.011
	TCP 114	7.17 $\pm$ 0.018	6.95 $\pm$ 0.027	0.22 $\pm$ 0.011
	TCP221	6.87 $\pm$ 0.007	6.75 $\pm$ 0.022	0.12 $\pm$ 0.007
	TCP 63	7.55 $\pm$ 0.018	7.37 $\pm$ 0.011	0.18 $\pm$ 0.011
	TCP 173	7.78 $\pm$ 0.018	7.06 $\pm$ 0.029	0.72 $\pm$ 0.025
	TCP 152	7.60 $\pm$ 0.011	7.41 $\pm$ 0.018	0.19 $\pm$ 0.011
	TCP 276	7.37 $\pm$ 0.011	7.29 $\pm$ 0.037	0.08 $\pm$ 0.011
	TCP 198	7.64 $\pm$ 0.014	7.47 $\pm$ 0.015	0.17 $\pm$ 0.014
	TCP 94	7.49 $\pm$ 0.014	7.37 $\pm$ 0.008	0.12 $\pm$ 0.015
	TCP 225	7.54 $\pm$ 0.014	6.92 $\pm$ 0.008	0.62 $\pm$ 0.011
	TCP 34	7.57 $\pm$ 0.018	7.49 $\pm$ 0.011	0.08 $\pm$ 0.011
Medium	TCP 58	7.90 $\pm$ 0.015	7.85 $\pm$ 0.012	0.05 $\pm$ 0.011
	TCP 93	7.78 $\pm$ 0.011	7.65 $\pm$ 0.025	0.13 $\pm$ 0.019
	TCP 175	7.91 $\pm$ 0.014	7.47 $\pm$ 0.011	0.44 $\pm$ 0.014
	TCP 224	7.91 $\pm$ 0.018	7.21 $\pm$ 0.018	0.70 $\pm$ 0.015
	TCP 40	7.64 $\pm$ 0.014	7.27 $\pm$ 0.011	0.37 $\pm$ 0.007
	TCP 139	7.57 $\pm$ 0.014	7.28 $\pm$ 0.021	0.29 $\pm$ 0.011
	TCP 84	8.26 $\pm$ 0.014	7.75 $\pm$ 0.021	0.51 $\pm$ 0.011
	TCP 137	7.90 $\pm$ 0.015	7.62 $\pm$ 0.045	0.28 $\pm$ 0.011
	TCP 64	7.61 $\pm$ 0.018	7.51 $\pm$ 0.029	0.10 $\pm$ 0.011
	TCP 202	7.60 $\pm$ 0.022	7.46 $\pm$ 0.027	0.14 $\pm$ 0.007
	TCP 240	7.56 $\pm$ 0.014	7.25 $\pm$ 0.033	0.31 $\pm$ 0.007
	TCP 12	7.70 $\pm$ 0.015	7.39 $\pm$ 0.007	0.31 $\pm$ 0.011
	TCP 194	7.67 $\pm$ 0.011	7.54 $\pm$ 0.007	0.13 $\pm$ 0.011
	TCP 222	7.69 $\pm$ 0.011	7.15 $\pm$ 0.014	0.54 $\pm$ 0.007
	TCP 247	7.84 $\pm$ 0.011	7.71 $\pm$ 0.011	0.13 $\pm$ 0.007
High	TCP 227	7.51 $\pm$ 0.018	7.40 $\pm$ 0.008	0.11 $\pm$ 0.011
	TCP 50	8.24 $\pm$ 0.018	7.92 $\pm$ 0.007	0.32 $\pm$ 0.011
	TCP 96	7.62 $\pm$ 0.011	7.28 $\pm$ 0.022	0.34 $\pm$ 0.007
	TCP 57	7.73 $\pm$ 0.015	7.46 $\pm$ 0.011	0.27 $\pm$ 0.011
	TCP 54	7.70 $\pm$ 0.018	7.44 $\pm$ 0.008	0.26 $\pm$ 0.007
	TCP 181	7.87 $\pm$ 0.011	7.57 $\pm$ 0.012	0.30 $\pm$ 0.011
	TCP 178	7.73 $\pm$ 0.011	7.46 $\pm$ 0.011	0.27 $\pm$ 0.007
	TCP 11	7.53 $\pm$ 0.015	7.45 $\pm$ 0.015	0.08 $\pm$ 0.007
	TCP 242	7.31 $\pm$ 0.018	7.26 $\pm$ 0.033	0.05 $\pm$ 0.011
	TCP 196	7.57 $\pm$ 0.018	7.45 $\pm$ 0.029	0.12 $\pm$ 0.011
	TCP 244	8.47 $\pm$ 0.011	8.08 $\pm$ 0.025	0.39 $\pm$ 0.014
	TCP 200	7.80 $\pm$ 0.019	7.70 $\pm$ 0.025	0.10 $\pm$ 0.007
	TCP 67	7.59 $\pm$ 0.018	7.19 $\pm$ 0.014	0.40 $\pm$ 0.011
	TCP 195	7.63 $\pm$ 0.021	7.54 $\pm$ 0.018	0.09 $\pm$ 0.007
	TCP 2	7.63 $\pm$ 0.018	7.33 $\pm$ 0.040	0.30 $\pm$ 0.007

*Average of three replicates

To investigate about the stability of curcumin in presence of different pH, equal concentration of curcumin (10 ppm) was tested for absorbance from lower to higher range of wavelengths. Degradation of standard curcumin with change of pH was graphically presented in Fig. 4.5. Curcumin itself exhibited maximum absorbance at 425 nm (1.170) when pH was set at 4.47. But when pH was changed from acidic to alkaline, the absorbance value showed decreasing trend and colour changed from light yellow to brick red (Plate 4.2). The maximum absorbance value was 1.101 at pH 6.1 (423 nm), 1.029 at pH 7.15 (419.50 nm), 0.839 at pH 8.71 (421 nm) and 0.791 at pH 10.8 (487 nm). So, it can be concluded that curcumin is degraded at alkaline pH because the colour is changed from light yellow to brick red and optical density value is decreased.

To investigate the effect of pH on rhizome colour development, the initial pH of aqueous solution of rhizome powder and final pH of the same after occasional stirring for 2 hours was measured (Table 4.21). The difference between initial pH and final pH was very low. Initial pH was found highest in TCP 244 (8.47) whereas lowest was recorded in TCP 100 (6.75) with 1.25 folds variation. TCP 244 also showed highest final pH (8.08) whereas lowest was found in TCP 138 (6.67) with 1.21 folds variation. Difference in pH was found highest in TCP 173 (0.72) whereas lowest was recorded in TCP 100, TCP 58 and TCP 242 (0.05) with 14.40 folds variation.

4.3.7 Reaction of curcumin with iron salt (FeCl_3) at different pH

In our earlier results we have observed that presence of high curcumin can be attributed to redness/deep orange colour of rhizome (Table 4.17). In another finding when we quantified the iron content from different genotype groups (Group I-III) it has exhibited significant positive correlation (0.618) with curcumin content. From our study we could also find out that curcumin stability is dependent on pH values and it is gradually converted to cationic forms which give brick red colour (Plate 4.2 and Fig. 4.5). To correlate all these findings we thought to investigate the binding property of iron salt (FeCl_3) with curcumin in presence of different pH.

FeCl_3 also showed maximum absorbance itself at 210 nm (1.857). But, absorbance value showed decreasing tendency when pH was changed from acidic to alkaline. The maximum absorbance value was 0.217 at pH 4.47 (397 nm), 0.186 at

pH 6.1 (374 nm), 0.310 at pH 7.15 (380.5 nm), 0.251 at pH 8.71 (379 nm) and 0.256 at pH 10.8 (458 nm) (Fig. 4.6).

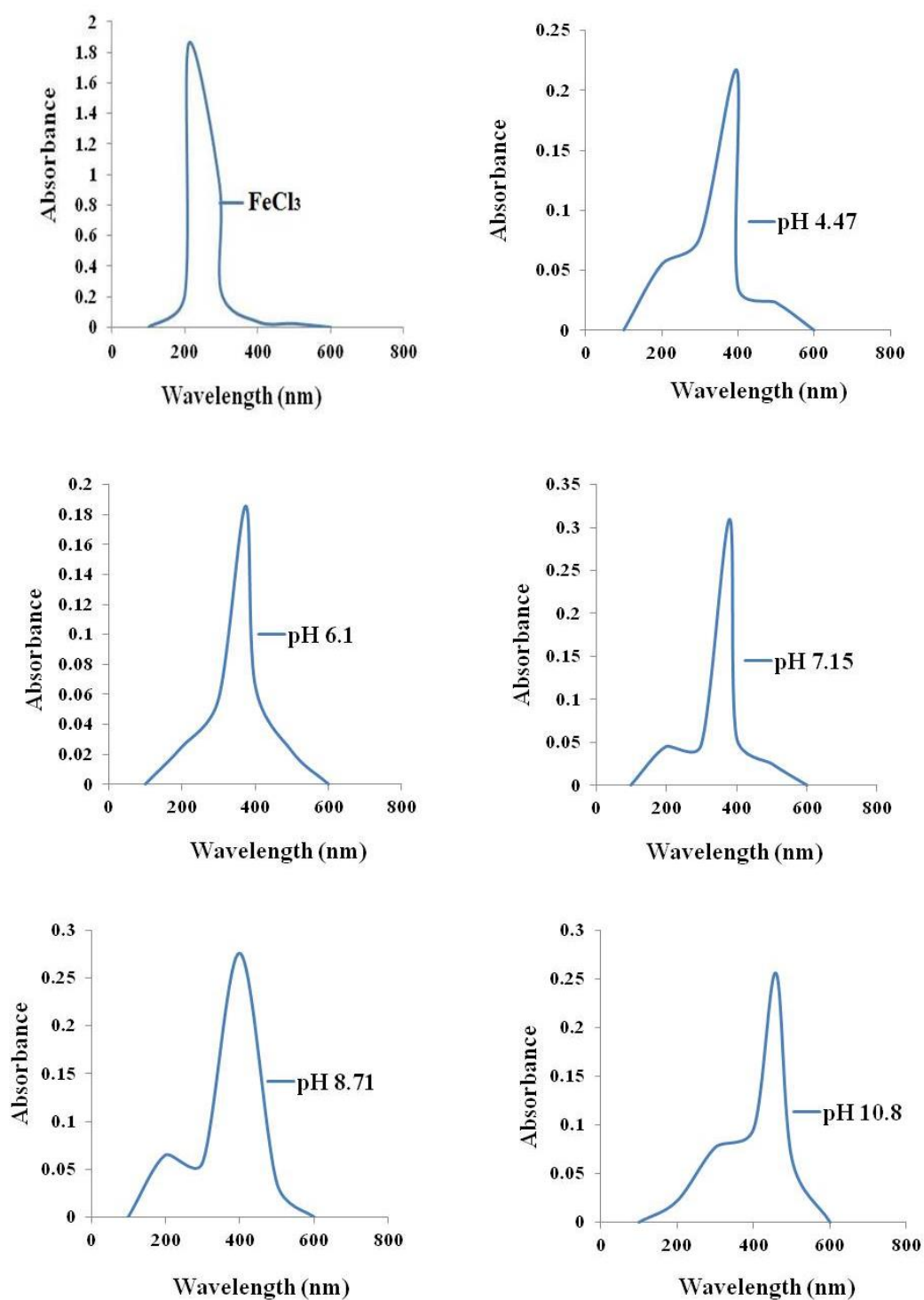


Fig. 4.6: Degradation of FeCl_3 with change of pH

When FeCl_3 solution (10 ppm) was reacted with standard curcumin, the result showed maximum absorbance at 431 nm (0.448). The pH was adjusted at 4.47. So, it was assumed that iron reacted with curcumin and formed complex molecule *i.e.* chelate form (Jiao *et al.*, 2006).

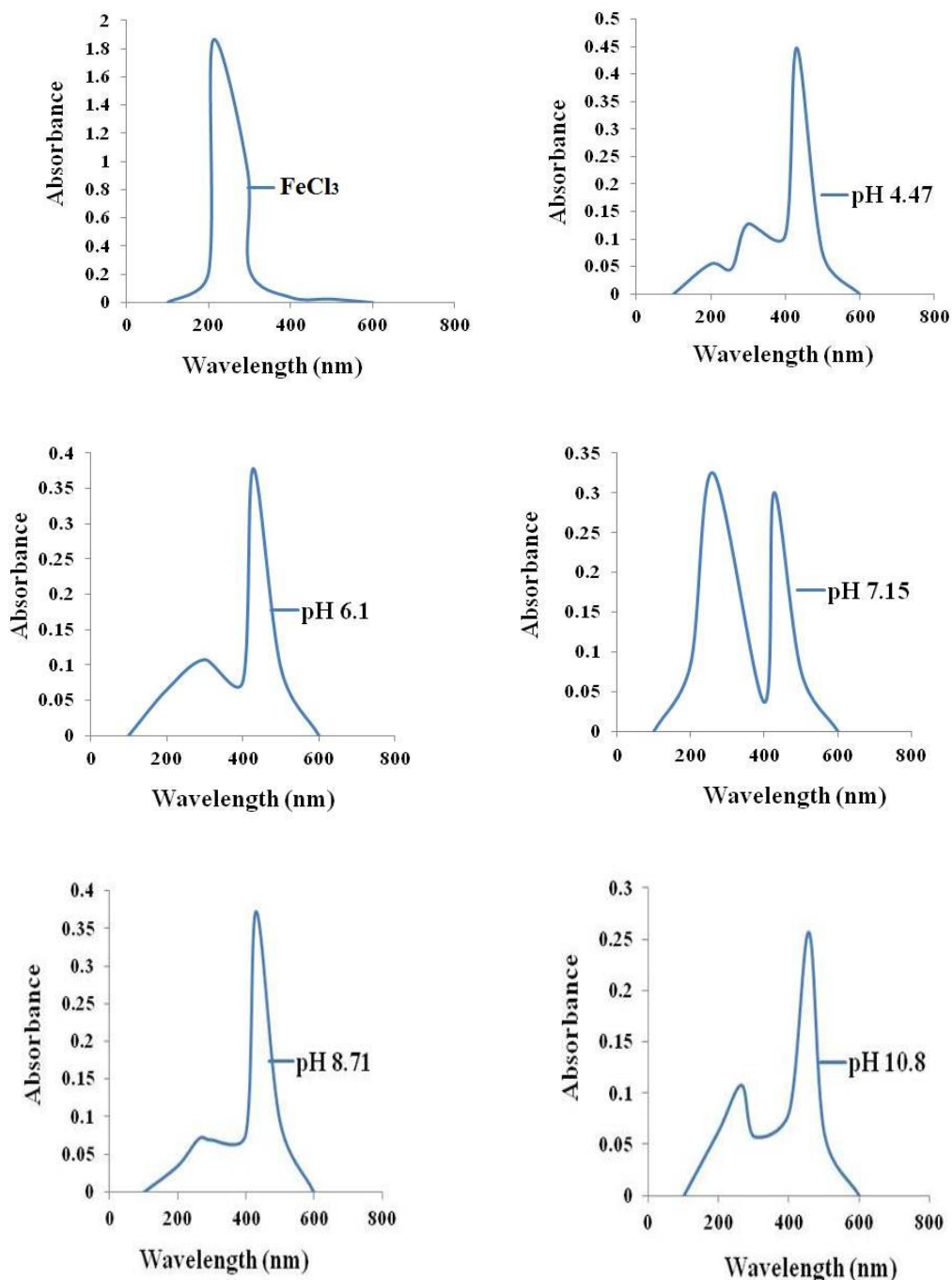


Fig. 4.7: Reaction of curcumin with FeCl_3 at different pH

Reaction of curcumin with FeCl_3 (10 ppm) at different pH was graphically presented in Fig. 4.7. FeCl_3 showed maximum absorbance itself at 210 nm (1.857).

When FeCl_3 reacted with curcumin at pH 4.47, the peak of FeCl_3 was shifted and maximum absorbance showed at 431 nm. The absorbance value of that point was 0.448. The same type of incident was seen at pH 7.15, 7.59, 8.71 and 10.8. At pH 7.15, the maximum absorbance value was 0.326 at 260.50 nm and 0.301 at 426 nm whereas at pH 7.59, the value was 0.396 at 429 nm. On the other hand, at pH 7.59 the maximum absorbance value was 0.396 at 429 nm whereas at pH 8.71, the maximum absorbance value was 0.372 at 430 nm. At pH 10.8, the maximum absorbance value was 0.257 at 456 nm. So, it can be concluded that curcumin reacts with FeCl_3 and forms complex chelate molecule (Jiao *et al.*, 2009). It seems that binding of iron salt to curcumin is maximum when the pH is in the range of 5-6. At higher pH iron changes its oxidation status. Hence, complex formation between curcumin and iron is also getting affected.

4.3.8 Correlation among the biochemical parameters

Correlation coefficient is a numerical measure of some type of correlation between two variables. It measures the strength of association between two variables and the direction of the relationship. The value of the correlation coefficient varies between +1 and -1.

Results of analysis revealed (Table 4.22) curcumin content was positively correlated with the amount of iron (0.618), total phenol (0.702), total protein (0.634), catalase (0.960), peroxidase (0.936), SOD (0.893), TAC (0.890), CUPRAC assay (0.940), a^* value (0.945), initial (0.414) and final (0.433) pH whereas negatively associated with DPPH (-0.888), ABTS (-0.883), L^* value (-0.654), b^* value (-0.492), hue angle (-0.921) and crude fat (-0.180).

It was observed that total phenol content was positively associated with total protein content (0.455), but this degree of association might not be the true picture as because amino acids like tyrosine interfere in the phenol estimation by FCR method (Bastola *et al.*, 2017) as well as phenolic compounds of varying structure (presence of double bonds, number of hydroxyl groups, presence of methyl groups, etc.) influence in the protein estimation by Lowry method (Niamke *et al.*, 2005). Total phenol content was also positively associated with iron (0.600), catalase (0.743), peroxidase (0.762), SOD (0.568), TAC (0.600), CUPRAC assay (0.736), a^* value (0.693), initial (0.375) and final (0.287) pH and difference in pH (0.221) but

negatively correlated with DPPH (−0.768), ABTS (−0.803), L* value (−0.798), b* value (−0.640) and hue angle (−0.769).

Iron content was positively correlated with the amount total protein (0.521), catalase (0.678), peroxidase (0.660), SOD (0.496), TAC (0.517), CUPRAC assay (0.513), a* value (0.530), initial (0.371) and final (0.424) pH while negatively associated with crude fat (−0.496), DPPH (−0.660), ABTS (−0.656), L* value (−0.541), b* value (−0.367) and hue angle (−0.539).

Total protein content was positively associated with catalase (0.663), peroxidase (0.547), SOD (0.286), TAC (0.598), CUPRAC assay (0.562), a* value (0.536), initial (0.286) and final (0.369) pH but negatively correlated with crude fat (−0.171), DPPH (−0.568), ABTS (−0.584), L* value (−0.454), b* value (−0.362) and hue angle (−0.540).

Crude fat was positively correlated with the amount of DPPH (0.299), ABTS (0.269), L* value (0.194), b* value (0.051) and hue angle (0.117) whereas negatively associated with catalase (−0.306), peroxidase (−0.226), SOD (−0.198), initial (−0.333) and final (−0.319) pH.

Catalase was positively associated with peroxidase (0.941), SOD (0.841), TAC (0.842), CUPRAC assay (0.906), a* value (0.909), initial (0.526) and final (0.517) pH while negatively correlated with DPPH (−0.931), ABTS (−0.919), L* value (−0.745), b* value (−0.549) and hue angle (−0.907).

Peroxidase was positively correlated with the amount of SOD (0.870), TAC (0.786), CUPRAC assay (0.909), a* value (0.912), initial (0.487) and final (0.494) pH but negatively associated with DPPH (−0.960), ABTS (−0.947), L* value (−0.797), b* value (−0.613) and hue angle (−0.932).

SOD was positively associated with TAC (0.736), CUPRAC assay (0.835), a* value (0.895), initial (0.392) and final (0.420) pH whereas negatively correlated with DPPH (−0.828), ABTS (−0.794), L* value (−0.554), b* value (−0.377) and hue angle (−0.849). DPPH was positively correlated with the amount of ABTS (0.975), L* value (0.822), b* value (0.623) and hue angle (0.906) while negatively associated with TAC (−0.724), CUPRAC assay (−0.875), a* value (−0.879), initial (−0.549) and final (−0.568) pH.

ABTS was positively associated with L* value (0.865), b* value (0.692) and hue angle (0.920) whereas negatively correlated with TAC (−0.755), CUPRAC assay (−0.889), a* value (−0.867), initial (−0.537) and final (−0.561) pH.

TAC was positively correlated with the amount of CUPRAC assay (0.877), a* value (0.838), initial (0.309) and final (0.352) pH but negatively associated with L* value (−0.533), b* value (−0.439) and hue angle (−0.817).

CUPRAC assay was positively associated with a* value (0.922), initial (0.436) and final (0.410) pH whereas negatively correlated with L* value (−0.743), b* value (−0.614) and hue angle (−0.944).

The L* value was positively correlated with b* value (0.893) and hue angle (0.808) while negatively associated with a* value (−0.655), initial (−0.611) pH, final (−0.527) pH and difference in pH (−0.260).

Table 4.22: Correlation among the studied biochemical parameters

	Cur-cumin	Phenol	Iron	Protein	Crude fat	Catalase	Peroxi-dase	SOD	DPPH	ABTS	TAC	CUP-RAC	L*	a*	b*	h	Initial pH	Final pH	pH Differ-ence
Curcumin	1	0.702**	0.618**	0.634**	-0.180*	0.960**	0.936**	0.893**	-0.888**	-0.883**	0.890**	0.940**	-0.654**	0.945**	-0.492**	-0.921**	0.414**	0.433**	0.044
Phenol		1	0.600**	0.455**	-0.124	0.743**	0.762**	0.568**	-0.768**	-0.803**	0.600**	0.736**	-0.798**	0.693**	-0.640**	-0.769**	0.375**	0.287**	0.221*
Iron			1	0.521**	-0.496**	0.678**	0.660**	0.496**	-0.660**	-0.656**	0.517**	0.513**	-0.541**	0.530**	-0.367**	-0.539**	0.371**	0.424**	-0.021
Protein				1	-0.171*	0.663**	0.547**	0.286**	-0.568**	-0.584**	0.598**	0.562**	-0.454**	0.536**	-0.362**	-0.540**	0.286**	0.369**	-0.090
Crude fat					1	-0.306**	-0.226**	-0.198*	0.299**	0.269**	-0.023	-0.062	0.194*	-0.138	0.051	0.117	-0.333**	-0.319**	-0.085
Catalase						1	0.941**	0.841**	-0.931**	-0.919**	0.842**	0.906**	-0.745**	0.909**	-0.549**	-0.907**	0.526**	0.517**	0.115
Peroxidase							1	0.870**	-0.960**	-0.947**	0.786**	0.909**	-0.797**	0.912**	-0.613**	-0.932**	0.487**	0.494**	0.079
SOD								1	-0.828**	-0.794**	0.736**	0.835**	-0.554**	0.895**	-0.377**	-0.849**	0.392**	0.420**	0.026
DPPH									1	0.975**	-0.724**	-0.875**	0.822**	-0.879**	0.623**	0.906**	-0.549**	-0.568**	-0.069
ABTS										1	-0.755**	-0.889**	0.865**	-0.867**	0.692**	0.920**	-0.537**	-0.561**	-0.059
TAC											1	0.877**	-0.533**	0.838**	-0.439**	-0.817**	0.309**	0.352**	-0.017
CUPRAC												1	-0.743**	0.922**	-0.614**	-0.944**	0.436**	0.410**	0.128
L*													1	-0.655**	0.893**	0.808**	-0.611**	-0.527**	-0.260**
a*														1	-0.465**	-0.961**	0.436**	0.434**	0.087
b*															1	0.683**	-0.503**	-0.452**	-0.179*
h																1	-0.502**	-0.486**	-0.122
Initial pH																	1	0.851**	0.442**
Final pH																		1	-0.086
pH Difference																			1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

The a^* value was positively associated with initial (0.436) pH and final (0.434) pH whereas negatively correlated with b^* value (−0.465) and hue angle (−0.961).

The b^* value was positively correlated with hue angle (0.683) but negatively associated with initial (−0.503) pH, final (−0.452) pH and difference in pH (−0.179).

Hue angle was negatively correlated with initial (−0.502) pH and final (−0.486) pH.

Initial pH was positively correlated with final (0.851) pH and difference in pH (0.442). There was no correlation between final pH and difference in pH.

From the correlation table, it can be assumed that increase in curcumin content influences the amount of iron, total phenol, total protein, catalase, peroxidase, superoxide dismutase, antioxidant activity and redness of the genotypes.

4.3.9 Heat map

It is a graphical representation of data where the individual values contained in a matrix are represented as colors. From the map (Fig. 4.8), it was observed that the green area contained the low curcumin containing genotypes whereas light green to white area covered the medium curcumin containing genotypes. High curcumin containing genotypes contained the light red to deep red area. The graph also showed that curcumin rich genotypes contained high amount of iron, total phenol, total protein, catalase, peroxidase, super oxide dismutase, antioxidant activity (TAC and CUPRAC) and redness (a^* value) which covered red colour in the graph. On the other hand, low curcumin containing genotypes gave less value of those parameter and showed green colour in the graph.

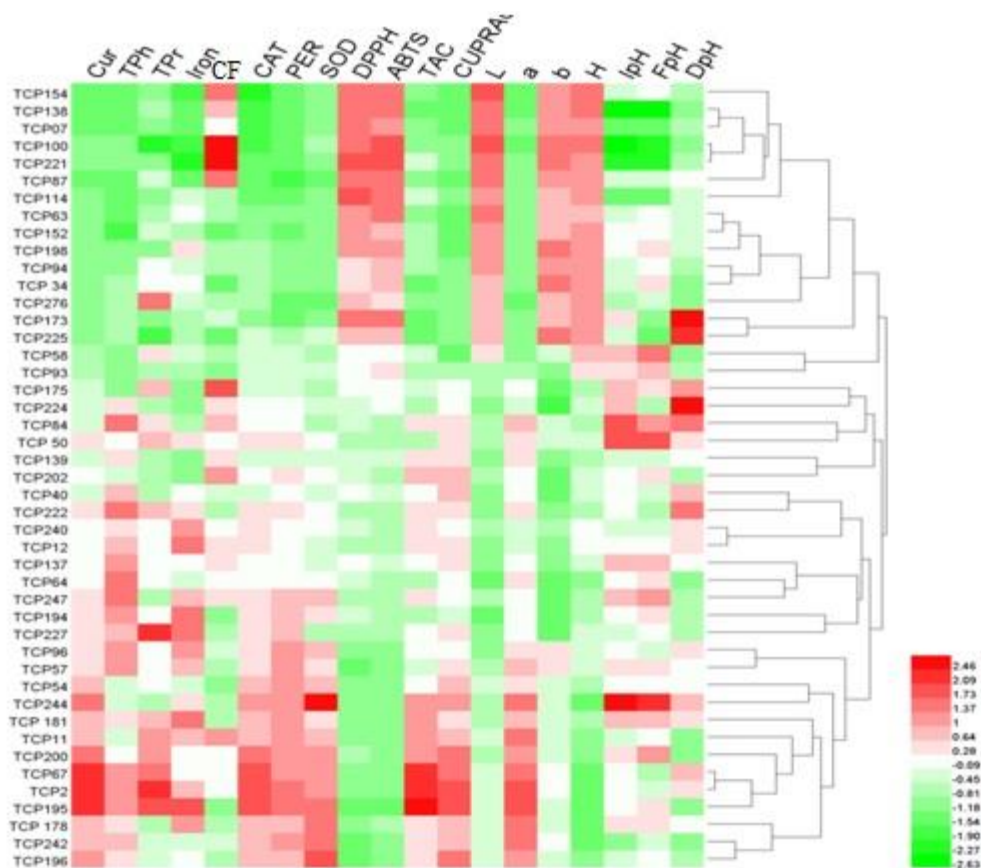


Fig. 4.8: Heat map of studied turmeric genotypes

(Cur- Curcumin content, Iron- Iron content, Tph- Total phenol, Tpr- Total protein, CAT- Catalase, PER- Peroxidase, SOD- Superoxide dismutase, CF- Crude fat, L- L* value, b- b* value, a- a* value, H- Hue angle, IpH- Initial pH, FpH- Final pH, DpH- Difference in pH)

4.3.10 Principal Component Analysis

Principal component analysis (PCA) is a technique that is useful for the classification and compression of data. Three principal components (PCs) accounted for 80.47 % of the variability among 45 turmeric genotypes. First principal component accounted for maximum 63.89 % of the total variation whereas second principal component contributed 9.27 % of total variance. Third principal component contributed 7.30 % of the variability present among the accessions.

The biochemical parameters which were contributed more positively to PC 1 included curcumin percentage (0.94), iron content (0.69), total phenol (0.80), total protein (0.62), catalase (0.96), peroxidase (0.96), super oxide dismutase (0.84), total antioxidant capacity (0.82), cupric ion reducing antioxidant capacity (0.93), a* value (0.92), initial pH (0.58), final pH (0.58) and difference in pH (0.12) whereas

negatively contributed to PC 1 included DPPH (-0.96), ABTS (-0.96), L* value (-0.84), b* value (-0.68), hue angle (-0.96) and crude fat (-0.25).

Table 4.23: Variation among turmeric germplasms accounted for first three principal components

Traits	PC 1	PC 2	PC 3
Curcumin	0.94	0.25	0.05
Total phenol	0.80	-0.01	-0.23
Total protein	0.62	0.10	0.27
Iron	0.69	-0.12	0.42
Crude fat	-0.25	0.47	-0.61
Catalase	0.96	0.07	0.09
Peroxidase	0.96	0.09	0.01
Superoxide dismutase	0.84	0.22	0.04
DPPH	-0.96	0.01	-0.05
ABTS	-0.96	0.01	-0.01
TAC	0.82	0.35	0.01
CUPRAC	0.93	0.20	-0.15
L* value	-0.84	0.29	0.26
a* value	0.92	0.23	-0.02
b* value	-0.68	0.29	0.36
Hue angle	-0.96	-0.11	0.14
Initial pH	0.58	-0.70	-0.03
Final pH	0.58	-0.50	0.30
Difference in pH	0.12	-0.48	-0.58
PV	63.89	9.27	7.30
CV	63.89	73.17	80.47

(PC- Principal component, PV- Percentage variance, CV- Cumulative variance)

PC 2 was associated positively with curcumin percentage (0.25), crude fat (0.47), super oxide dismutase (0.22), total antioxidant capacity (0.35), cupric ion reducing antioxidant capacity (0.20), L* value (0.29), a* value (0.23), b* value (0.29) while negatively associated with initial pH (-0.70), final pH (-0.50) and difference in pH (-0.48).

PC 3 was correlated positively with total protein (0.27), iron content (0.42), L* value (0.26), b* value (0.36), final pH (0.30) whereas negatively correlated with total phenol (-0.23), crude fat (-0.61) and difference in pH (-0.58) (Table 4.23). From the table, it can be concluded that PC 1 is the most important principal component among three principal components because almost all parameters are strongly and positively contributed.

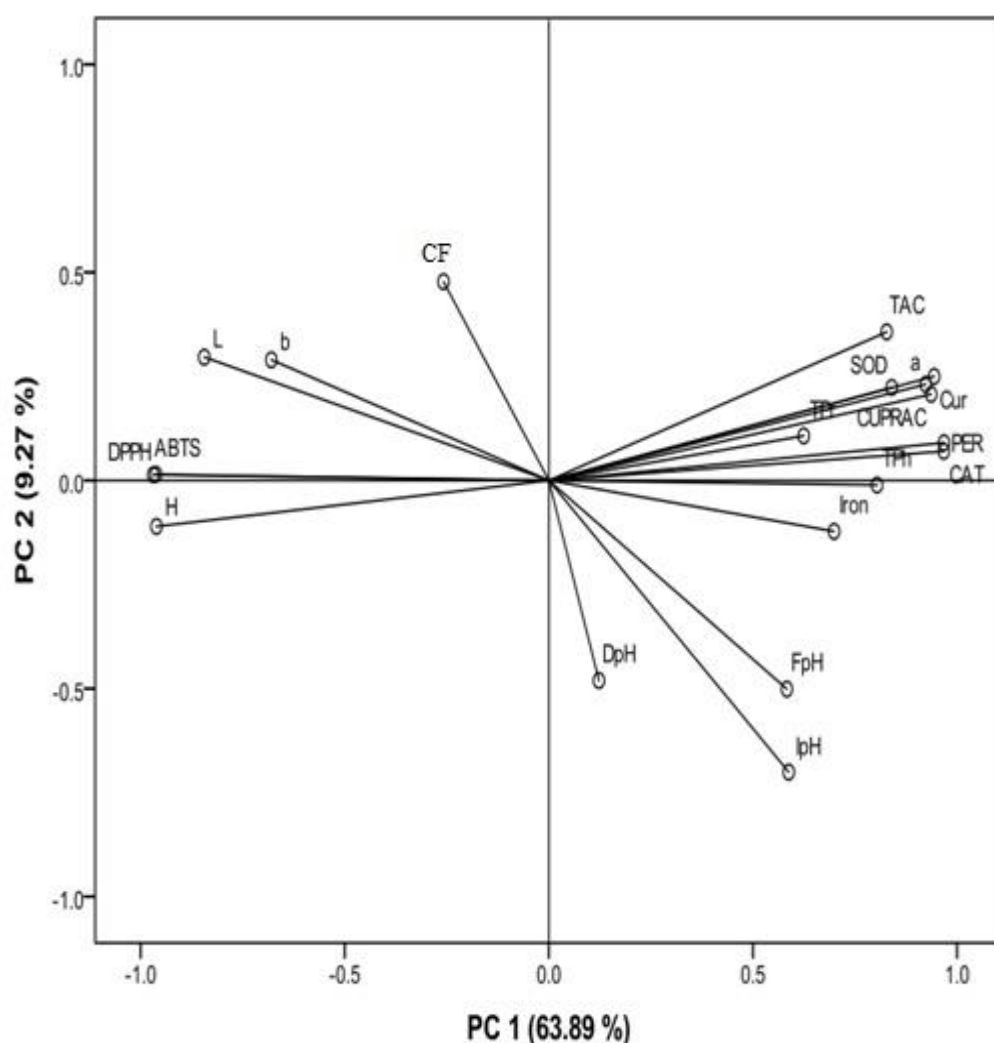


Fig. 4.9: Variables factor map of first two principal components showing the contribution of various traits based on mean values in the separation of various genotypes of turmeric

(Cur- Curcumin content, Iron- Iron content, Tph- Total phenol, Tpr- Total protein, CAT- Catalase, PER- Peroxidase, SOD- Superoxide dismutase, CF- Crude fat, L- L* value, b- b* value, a- a* value, H- Hue angle, IpH- Initial pH, FpH- Final pH, DpH- Difference in pH)

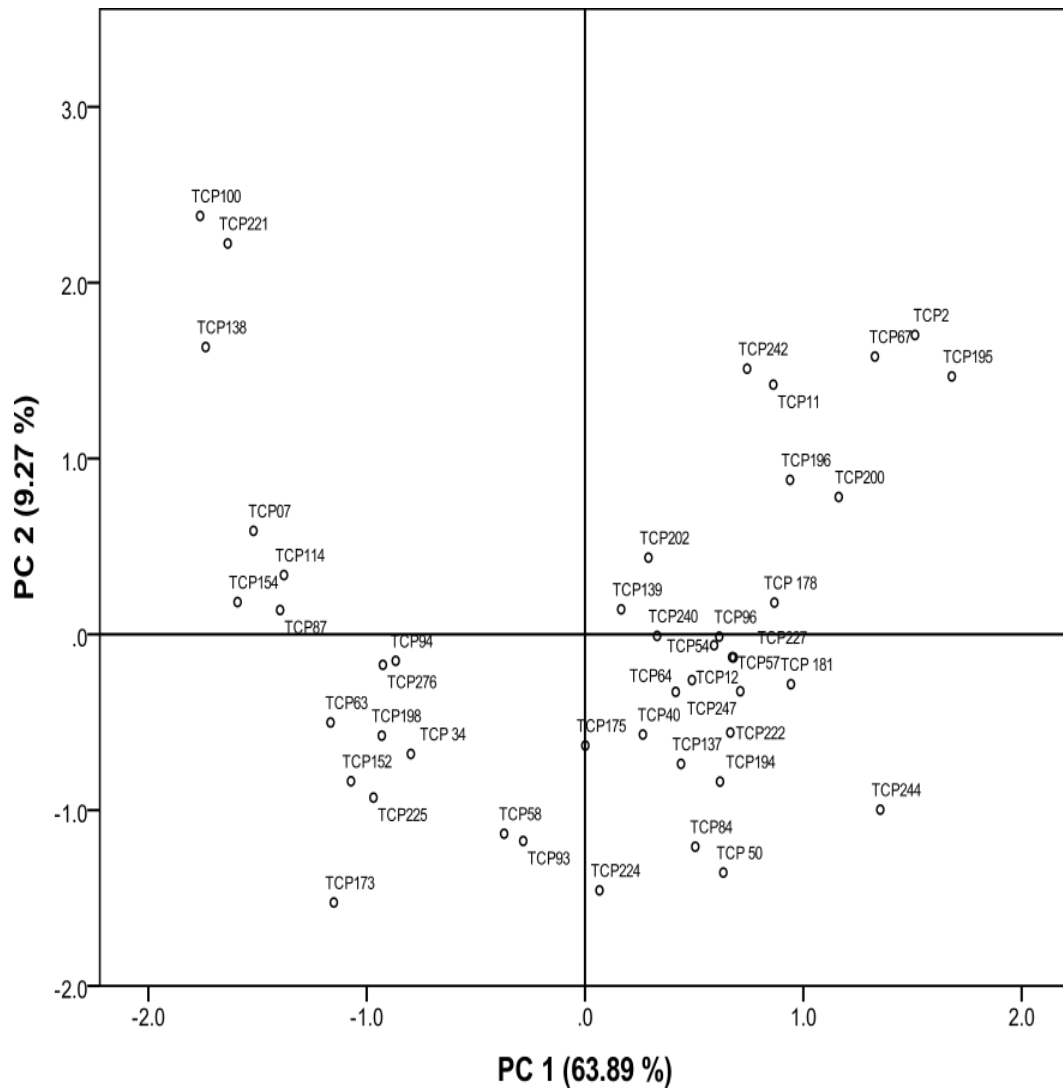


Fig. 4.10: Individuals factor map of first two principal components (PC) based on mean values of nineteen quantitative traits in 45 genotypes of turmeric

The relationship between parameters was shown in Fig. 4.9 whereas relationship among the genotypes was presented in Fig. 4.10. The figures showed clearly about the genotypes and traits. Curcumin content was positively correlated with the amount of iron (0.618), total phenol (0.702), total protein (0.634), catalase (0.960), peroxidase (0.936), SOD (0.893), TAC (0.890), CUPRAC assay (0.940), a^* value (0.945), initial (0.414) and final (0.433) pH. So, these parameters were presented at the right side of the biplot. On the other hand, curcumin content was negatively correlated with DPPH (-0.888), ABTS (-0.803), L^* value (-0.654), b^* value (-0.492), hue angle (-0.921) and crude fat (-0.180). So, these parameters were presented at the left side of the biplot.

4.4 Standardization of protocol for micropropagation

The third objective of the present research programme was standardization of protocol for micropropagation of best of the selected cultivars. Result of experiments conducted under objective 1 and 2 with forty five genotypes of *Curcuma longa* have concluded that TCP 2 is the best genotype among the genotypes considering its yield, rhizome size, colour and other biochemical parameter. So, in the present objective TCP 2 was considered for the standardization of micropropagation technique.

On the other hand, *Curcuma caesia* is one of the endangered medicinally important herb that has not been investigated thoroughly for its biochemical potential till date. The conservation and mass multiplication of this important herb has been addressed in the present objective using the micropropagation techniques. So, in the present study, the purpose of the investigation was to develop a suitable technique for more appropriate clonal propagation of *C. longa* (TCP 2) and black turmeric (*C. caesia*) in a cost effective manner for getting disease free and homogeneous planting material for off season and whole year cultivation for the benefit of the farmers.

For developing tissue culture protocol for any plant, different growth regulators in different combinations are supplemented with basal medium (usually MS medium) and their effects are tested on four aspects of plant growth namely shoot bud induction, multiple shoot regeneration, leaf emergence and root initiation and ability of callus induction by different plant parts are also observed. The same was followed in the present programme and results were observed as recorded below.

4.4.1 Micropropagation of biochemically superior genotype TCP 2 (*Curcuma longa* L.)

4.4.1.1 Effect of growth regulators on shoot bud induction of TCP 2 (*C. longa*)

The present investigation was carried out to determine the *in vitro* efficacy of different auxins and cytokinins (BAP, NAA and Kinetin) on shoot bud induction of TCP 2 (*C. longa*). The basal medium used for shoot bud initiation purpose was MS media with different hormonal combinations as summarized in Table 4.24.

The rhizome buds culture resulted that MS medium with 5.0 mg/L BAP and 1.0 mg/L NAA combination responded best (60.33%) followed by 5.0 mg/L BAP and 2.0 mg/L NAA combination (59.33%). The shoot buds initiated in these treatments within 20 days and 22 days respectively. On the other hand, the treatment of 4.0 mg/L BAP + 2.0 mg/L Kinetin also responded promisingly in respect of the days of initiation of shoot bud (22.67) but showed moderate response in respect of shoot bud induction percentage (58.67%). It was also observed that moderate response was found with the medium containing treatments like 0.5 mg/L BAP + 0.5 mg/L Kinetin, 1.0 mg/L BAP + 0.5 mg/L NAA, 2.0 mg/L BAP + 0.5 mg/L NAA, 0.5 mg/L BAP + 0.5 mg/L Kinetin, 1.0 mg/L BAP + 0.5 mg/L Kinetin, 2.0 mg/L BAP + 1.0 mg/L Kinetin and 3.0 mg/L BAP + 1.0 mg/L Kinetin whereas lowest was observed in full strength MS medium without hormone (34.67%). There were no significant difference within these four different hormonal supplementations (4.0 mg/L BAP + 1.0 mg/L NAA, 4.0 mg/L BAP + 2.0 mg/L NAA, 5.0 mg/L BAP + 1.0 mg/L NAA and 5.0 mg/L BAP + 2.0 mg/L NAA) because these hormonal combinations grouped into same subset (subset a). Maximum number of multiple shoot induction was observed after 9 weeks of culture. From the table, it was evident that out of seventeen different combinations, rhizome buds showed best performance in medium containing 5.0 mg/L BAP + 1.0 mg/L NAA in terms of shoot bud induction percentage (60.33). Effect of BAP, NAA and Kinetin on days for multiple shoot initiation and shoot bud induction percentage in TCP 2 (*C. longa*) has been graphically presented in Fig. 4.11 and Fig. 4.12.

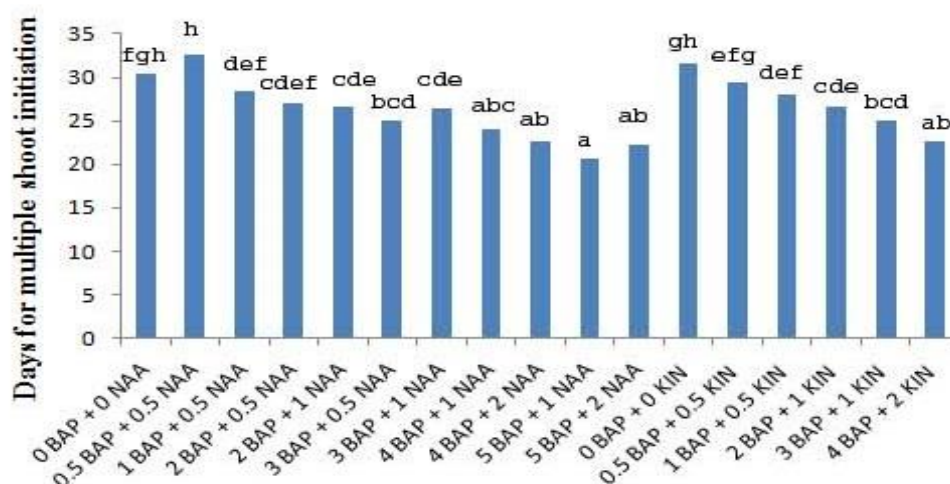
In the present investigation, it was observed that with increase in the concentration of BAP, the rate of shoot bud induction increased.

Earlier among the different treatments of cytokinin and auxin tested, the best response ($81.66 \pm 4.84\%$) in shoot bud induction was found in presence of 2.5 mg/L BA and 1.5 mg/L NAA after 22 days of incubation (Ghosh *et al.*, 2013). In another study, 0.1 mg/L kinetin and 0.2 mg/L BAP was the best combination for shoot formation. The combination of 0.1 mg/L kinetin and 0.2 mg/L BAP was best resulting in formation of 7-8 healthy green shoots with 80% survival rate in the field (Nadgauda *et al.*, 1978). So the results of the present study were in conformity with the earlier ones.

Table 4.24: Effect of BAP, NAA and Kinetin on *in vitro* shoot bud induction of TCP 2 (*C. longa*)

Hormone concentration (mg/L)			Days for multiple shoot initiation (mean* $\pm$ SE)	Shoot bud induction (%) (mean* $\pm$ SE)
BAP	NAA	Kinetin		
0.0	0.0	-	30.33 $\pm$ 1.080 ^{fgh}	36.67 $\pm$ 2.160 ^f
0.5	0.5	-	32.67 $\pm$ 1.472 ^h	42.33 $\pm$ 0.816 ^e
1.0	0.5	-	28.33 $\pm$ 1.472 ^{def}	41.00 $\pm$ 1.225 ^e
2.0	0.5	-	27.00 $\pm$ 0.707 ^{cdef}	50.67 $\pm$ 1.472 ^d
2.0	1.0	-	26.67 $\pm$ 0.816 ^{cde}	55.33 $\pm$ 1.080 ^{bc}
3.0	0.5	-	25.00 $\pm$ 1.225 ^{bcd}	57.00 $\pm$ 0.707 ^{ab}
3.0	1.0	-	26.33 $\pm$ 1.780 ^{cde}	56.67 $\pm$ 1.780 ^{ab}
4.0	1.0	-	24.00 $\pm$ 1.871 ^{abc}	59.00 $\pm$ 2.121 ^{ab}
4.0	2.0	-	22.67 $\pm$ 2.160 ^{ab}	58.67 $\pm$ 2.041 ^{ab}
5.0	1.0	-	20.67 $\pm$ 0.408 ^a	60.33 $\pm$ 0.408 ^a
5.0	2.0	-	22.33 $\pm$ 1.080 ^{ab}	59.33 $\pm$ 1.472 ^a
0.0	-	0.0	31.67 $\pm$ 1.080 ^{gh}	34.67 $\pm$ 1.780 ^f
0.5	-	0.5	29.33 $\pm$ 1.780 ^{efg}	40.33 $\pm$ 1.472 ^e
1.0	-	0.5	28.00 $\pm$ 1.225 ^{def}	43.67 $\pm$ 1.633 ^e
2.0	-	1.0	26.67 $\pm$ 0.816 ^{cde}	48.67 $\pm$ 1.080 ^d
3.0	-	1.0	25.00 $\pm$ 0.707 ^{bcd}	52.00 $\pm$ 1.414 ^{cd}
4.0	-	2.0	22.67 $\pm$ 0.408 ^{ab}	55.33 $\pm$ 0.816 ^{bc}

*Average of three replicates

**Fig. 4.11.** Effect of BAP, NAA and Kinetin on days for multiple shoot initiation in TCP 2 (*C. longa*)

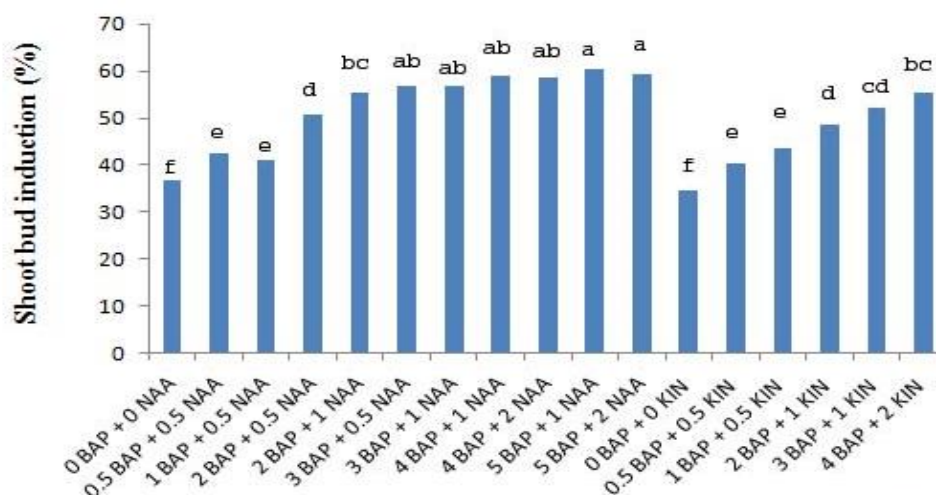


Fig. 4.12. Effect of BAP, NAA and Kinetin on shoot bud induction percentage in TCP 2 (*C. longa*)

4.4.1.2 Effect of different growth regulators on multiple shoot regeneration and leaf emergence of TCP 2 (*C. longa*)

After initiation of shoot, the media composition was changed for multiple shoot proliferation. The basal medium was MS medium with different hormonal supplementations as given in Table 4.25.

The rhizome bud culture in MS medium showed (Table 4.25) that the medium supplemented with 5.0 mg/L BAP + 2.0 mg/L NAA produced highest shoot multiplication (4.00 shoots/explant) followed by 5.0 mg/L BAP + 1.0 mg/L NAA (3.67 shoots/explant) and 3.0 mg/L BAP + 1.0 mg/L Kinetin (3.33 shoots/explant) within 9 weeks of culture whereas lowest was found in full strength MS medium without hormone (1.00 shoots/explant).

A moderate response in terms of number of shoots/explant was found in the media containing the combinations 2.0 mg/L BAP + 0.5 mg/L NAA, 2.0 mg/L BAP + 1.0 mg/L NAA, 3.0 mg/L BAP + 0.5 mg/L NAA, 3.0 mg/L BAP + 1.0 mg/L NAA, 4.0 mg/L BAP + 1.0 mg/L NAA, 4.0 mg/L BAP + 2.0 mg/L NAA, 2.0 mg/L BAP + 1.0 mg/L Kinetin and 4.0 mg/L BAP + 2.0 mg/L Kinetin. There were no significant difference among the different treatments except 0.5 mg/L BAP + 0.5 mg/L Kinetin, 1.0 mg/L BAP + 0.5 mg/L NAA, 2.0 mg/L BAP + 0.5 mg/L NAA, 0.5 mg/L BAP + 0.5 mg/L Kinetin, 1.0 mg/L BAP + 0.5 mg/L Kinetin and full strength MS medium without hormone because almost all the treatments appeared into the same subset (subset e). With an increase in the concentration of BAP, the number of shoots per explant increased. Out of seventeen different treatments,

rhizome buds showed best performance in 5.0 mg/L BAP + 2.0 mg/L NAA containing medium.

Table 4.25: Effect of BAP, NAA and Kinetin on *in vitro* multiplication of number of shoots/explant and number of leaves/shoot of TCP 2 (*C. longa*)

Hormone concentration (mg/L)			No. of shoots/ explant (mean* ± SE)	No. of leaves/ shoot (mean* ± SE)
BAP	NAA	Kinetin		
0.0	0.0	-	1.33 ± 0.408 ^{de}	2.00 ± 0.707 ^{bc}
0.5	0.5	-	1.33 ± 0.408 ^{de}	2.00 ± 0.000 ^{bc}
1.0	0.5	-	1.67 ± 0.816 ^{cde}	2.33 ± 0.408 ^{bc}
2.0	0.5	-	2.00 ± 0.707 ^{bcde}	2.67 ± 1.080 ^{bc}
2.0	1.0	-	2.33 ± 1.080 ^{abcde}	2.33 ± 0.408 ^{bc}
3.0	0.5	-	2.33 ± 0.408 ^{abcde}	2.67 ± 0.816 ^{bc}
3.0	1.0	-	2.67 ± 0.816 ^{abcde}	2.00 ± 0.707 ^{bc}
4.0	1.0	-	2.67 ± 0.408 ^{abcde}	2.67 ± 0.408 ^{bc}
4.0	2.0	-	3.00 ± 0.707 ^{abcd}	4.33 ± 0.408 ^a
5.0	1.0	-	3.67 ± 0.816 ^{ab}	2.67 ± 0.816 ^{bc}
5.0	2.0	-	4.00 ± 0.707 ^a	3.33 ± 0.408 ^{ab}
0.0	-	0.0	1.00 ± 0.000 ^e	2.00 ± 0.707 ^{bc}
0.5	-	0.5	1.33 ± 0.408 ^{de}	1.33 ± 0.408 ^c
1.0	-	0.5	1.67 ± 0.816 ^{cde}	2.33 ± 0.408 ^{bc}
2.0	-	1.0	2.00 ± 0.707 ^{abcd}	3.33 ± 0.408 ^{ab}
3.0	-	1.0	3.33 ± 0.408 ^{abc}	2.67 ± 0.816 ^{bc}
4.0	-	2.0	2.33 ± 0.408 ^{abcde}	2.67 ± 0.408 ^{bc}

*Average of three replicates

Earlier it has been reported that higher concentration of cytokinin has a positive effect on shoot proliferation of some species of *Zingiberaceae* (Ikeda and Tambe, 1989 and Balachandran *et al.*, 1990). The present study also reflected the positive effect of BAP on shoot bud initiation. From the present study it is clear that the low amount of NAA along with cytokinin induces better shoot bud initiation. Effect of BAP, NAA and Kinetin on number of shoots/explant in TCP 2 (*C. longa*) has been graphically presented in Fig. 4.13.

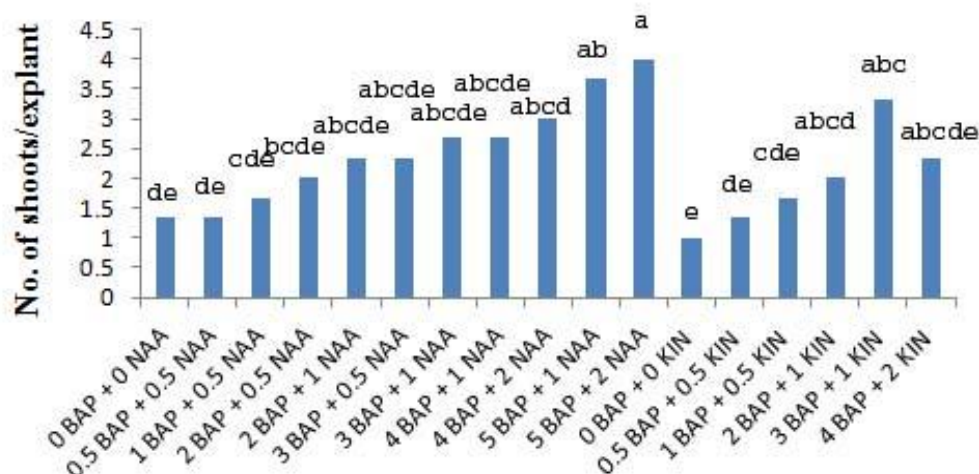


Fig. 4.13. Effect of BAP, NAA and Kinetin on number of shoots/explant in TCP 2 (*C. longa*)

The rhizome bud culture in MS medium in terms of number of leaves/shoot showed that MS medium with 4.0 mg/L BAP + 2.0 mg/L NAA (4.33) responded best followed by 5.0 mg/L BAP + 2.0 mg/L NAA and 2.0 mg/L BAP+ 1.0 mg/L Kinetin (3.33) whereas lowest was observed in 0.5 mg/L BAP and 0.5 mg/L Kinetin combination (1.33). On the other hand, moderate response was found in 1.0 mg/L BAP+ 0.5 mg/L NAA, 2.0 mg/L BAP+ 0.5 mg/L NAA, 2.0 mg/L BAP+ 1.0 mg/L NAA, 3.0 mg/L BAP+ 0.5 mg/L NAA, 4.0 mg/L BAP+ 1.0 mg/L NAA, 5.0 mg/L BAP+ 1.0 mg/L NAA, 1.0 mg/L BAP+ 0.5 mg/L Kinetin, 3.0 mg/L BAP+ 1.0 mg/L Kinetin and 4.0 mg/L BAP+ 2.0 mg/L Kinetin containing medium. There were no significant difference among the different treatments except 4.0 mg/L BAP + 2.0 mg/L NAA, 5.0 mg/L BAP + 2.0 mg/L NAA and 2.0 mg/L BAP + 1.0 mg/L Kinetin containing medium because almost all the treatments grouped into same subset (subset a). Out of seventeen different hormonal supplementations, rhizome buds showed best performance in producing number of leaves/shoot in medium containing 4.0 mg/L BAP + 2.0 mg/L NAA (Table 4.25). The effect of BAP, NAA and Kinetin on number of leaves/shoot in TCP 2 (*C. longa*) has been presented graphically in Fig. 4.14.

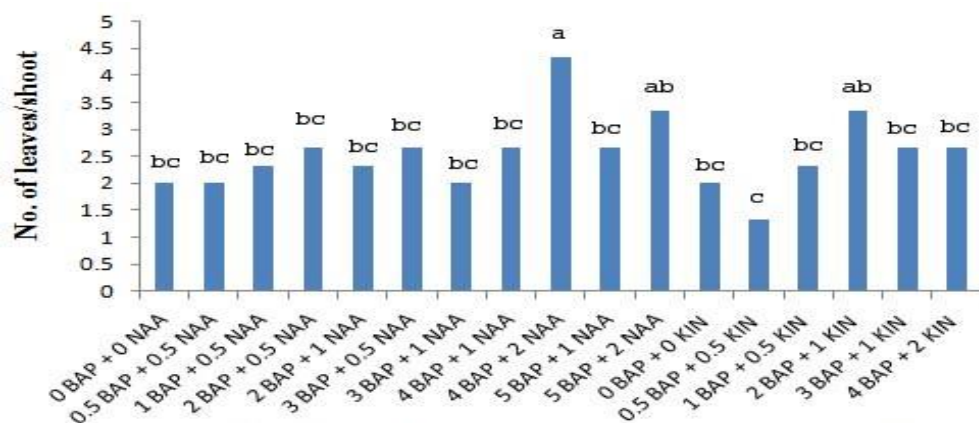


Fig. 4.14. Effect of BAP, NAA and Kinetin on number of leaves/shoot in TCP 2 (*C. longa*)

In the present study, it was found that with increase in the concentration of BAP, the rate of shoot multiplication increased. But, BAP and NAA combination showed better result than BAP alone. Similar type findings were reported by Sharma and Singh (1997) and Prathanturarug *et al.* (2003).

According to Prathanturarug *et al.* (2003), high frequency of shoot multiplication was observed in *C. longa* using thidiazuron as growth hormone. In this study, 3.42 to 5.08 shoots from undivided buds and 3.47 to 4.36 shoots from divided buds were obtained and after 12 weeks, maximum number of shoots (18.22 ± 0.62) was formed. In another study, MS medium containing 5.0 mg/L BAP + 2.0 mg/L NAA and 3.0 mg/L BAP + 0.5 mg/L NAA gave nearly 5.65 shoots per explant. The longest shoot length (9.64) was found on the media containing 1.0 mg/L of BAP + 2.0 mg/L of NAA and 5.0 mg/L of BAP + 2.0 mg/L of NAA respectively (Ikeda and Tambe, 1989). So the results of this study are in consonance with the earlier findings.

4.4.1.3 Effect of different growth regulators on root initiation of TCP 2 (*C. longa*)

The well developed shoots obtained from earlier study were taken from shoot clumps and transferred to root induction medium i.e. full strength MS medium containing different phytohormone concentrations as summarized in Table 4.26. Here, IBA is the phytohormone primarily responsible for initiation of roots. It was observed that different combinations had different effects in producing roots of TCP 2 (*C. longa*).

Table 4.26: Effect of BAP, NAA, Kinetin and IBA on *in vitro* multiplication of number of roots/shoot of TCP 2 (*C. longa*)

Hormone concentration (mg/L)				No. of roots/ shoot (mean* $\pm$ SE)
BAP	NAA	Kinetin	IBA	
0.0	0.0	-	0.0	3.00 $\pm$ 0.707 ^{fg}
0.0	0.5	-	0.5	4.00 $\pm$ 0.000 ^{defg}
1.0	0.0	-	1.0	2.67 $\pm$ 1.080 ^g
0.0	0.0	-	1.0	5.00 $\pm$ 0.707 ^{cdef}
1.0	0.0	-	1.5	3.67 $\pm$ 0.408 ^{efg}
0.0	0.5	-	1.5	4.67 $\pm$ 1.472 ^{cdefg}
0.0	0.0	-	1.5	8.33 $\pm$ 0.816 ^b
1.0	0.0	-	1.5	5.33 $\pm$ 0.408 ^{bcde}
0.0	0.0	-	2.0	8.67 $\pm$ 0.408 ^a
0.0	0.5	-	2.0	5.67 $\pm$ 0.408 ^{bcde}
1.0	0.0	-	2.0	6.00 $\pm$ 1.225 ^{bcd}
0.0	-	0.0	0.0	2.67 $\pm$ 1.080 ^g
0.0	-	0.0	1.0	6.67 $\pm$ 0.408 ^{abc}
0.0	-	0.5	1.0	6.00 $\pm$ 1.225 ^{bcd}
0.0	-	0.0	2.0	7.33 $\pm$ 0.408 ^{ab}
1.0	-	0.0	2.0	4.67 $\pm$ 0.816 ^{cdefg}
0.0	-	0.5	2.0	5.00 $\pm$ 0.707 ^{cdef}

*Average of three replicates

MS medium supplemented with a combination of 2.0 mg/L IBA gave the maximum number (8.67) of roots/shoot followed by 1.5 mg/L IBA (8.33 roots/shoot) while lowest number of roots/shoot (2.67) was found in full strength MS medium without hormone and 1.0 mg/L BAP + 1.0 mg/L IBA containing medium (Table 4.26). On the other hand, moderate response was found in 0.5 mg/L NAA + 0.5 mg/L IBA, 1.0 mg/L IBA, 0.5 mg/L NAA + 1.5 mg/L IBA, 1.0 mg/L BAP + 1.5 mg/L IBA, 0.5 mg/L NAA + 2.0 mg/L IBA, 1.0 mg/L BAP + 2.0 mg/L IBA and 0.5 mg/L Kinetin + 1.0 mg/L IBA containing medium. There was no significant difference found within these two different hormonal supplementations (1.0 mg/L IBA and 2.0 mg/L IBA) because these hormonal combinations grouped into same subset (subset a). Out of seventeen different hormonal supplementations, rhizome buds showed best performance in 2.0 mg/L IBA containing medium in terms of number of roots/shoot. The effect of BAP, NAA, Kinetin and IBA on *in vitro* multiplication of number of roots/shoot of TCP 2 (*C. longa*) has been graphically given in Fig. 4.15.

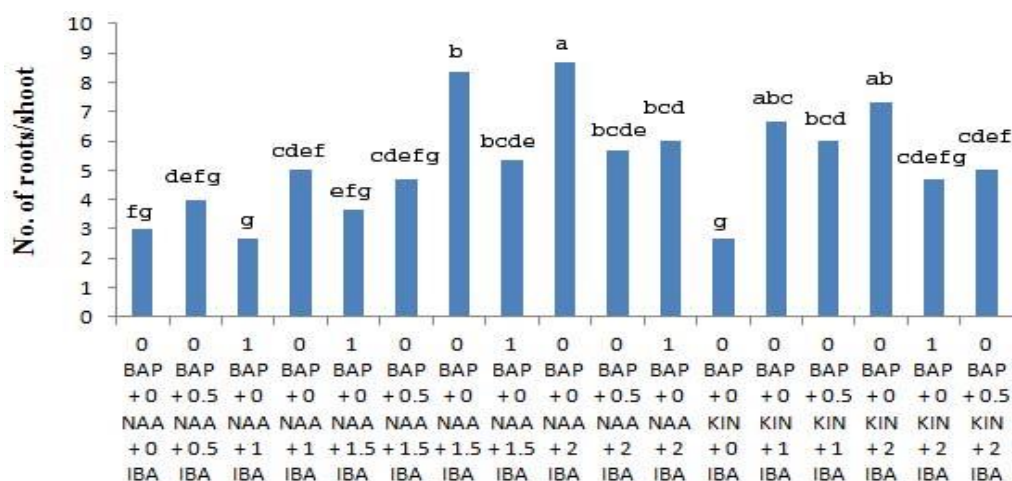


Fig. 4.15. Effect of BAP, NAA, Kinetin and IBA on number of roots/shoot in TCP 2 (*C. longa*)

Root initials were developed into a good root system within 25-30 days. After hardening plantlets were kept in greenhouse and transferred to the field. The survival rate of developed plantlets under field condition was 60-65%.

Shahinozzaman *et al.* (2013) reported that environmental factors such as light, temperature and humidity were the essential parameters for production of multiple shoots and the success of micropropagation was greatly influenced by the composition of the culture medium, 16 h photoperiod at 3000 lux, 8 hours dark period, $25 \pm 2^\circ\text{C}$ temperature and 70-80% relative humidity.

In the present investigation, it was observed that 2.0 mg/L IBA was optimum for the development of adequate number of roots/shoot. The present study revealed that shoot bud induction, number of shoots/explant, number of leaves/shoot and number of roots/shoot of TCP 2 (*C. longa*) were depended on the growth regulators used. Similar types of results were reported by Rout *et al.* (1995) and Ghosh *et al.* (2013).

4.4.1.4 Effect of growth regulators on callus induction in TCP 2 (*C. longa*)

Callus induction by inoculating leaf tissue (blade discs, sheath base and sheath top) was achieved using different combinations of 2,4-D along with Kinetin (Table 4.27). Callus induction started after 25 days and maximum callus growth was obtained after 142.67 days in the medium containing MS basal salts supplemented with 2.0 mg/L of 2,4-D (21.33%) followed by medium containing 1.0 mg/L 2,4-D (18.33%) and 1.0 mg/L 2,4-D + 2.0 mg/L kinetin (18.33%).

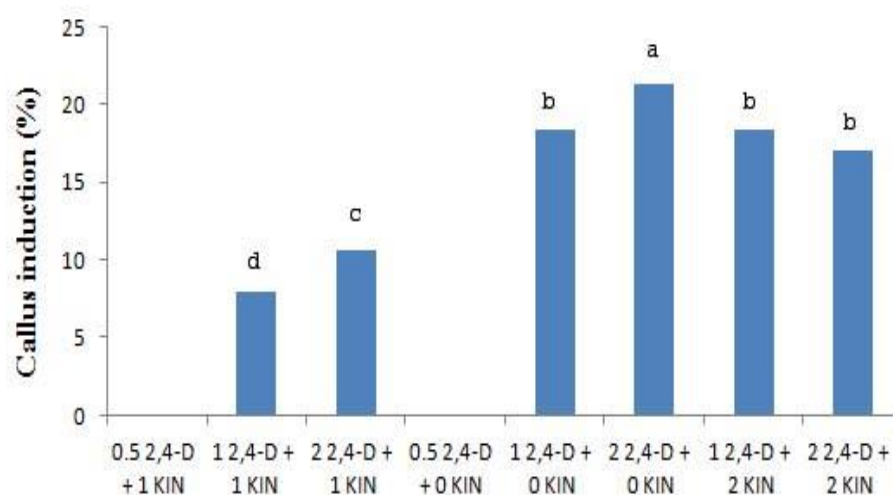
Table 4.27: Effect of 2, 4-D and Kinetin on the induction of callus in TCP 2 (*C. longa*)

Hormone concentration (mg/L)		Callus induction (%) (mean* $\pm$ SE)	Day of callus initiation (mean* $\pm$ SE)	Callus growth
2, 4- D	Kinetin			
0.5	1.0	0.00	-	-
1.0	1.0	8.00 $\pm$ 0.707 ^d	164.00 $\pm$ 1.414 ^e	+
2.0	1.0	10.67 $\pm$ 0.816 ^c	153.33 $\pm$ 1.472 ^{cd}	+
0.5	0.0	0.00	-	-
1.0	0.0	18.33 $\pm$ 0.816 ^b	151.33 $\pm$ 0.816 ^c	++
2.0	0.0	21.33 $\pm$ 0.408 ^a	142.67 $\pm$ 0.408 ^a	+++
1.0	2.0	18.33 $\pm$ 1.080 ^b	155.67 $\pm$ 1.780 ^d	++
2.0	2.0	17.00 $\pm$ 1.225 ^b	147.67 $\pm$ 1.080 ^b	++

*Average of three replicates

Callus growth was relatively poor in the medium containing 1.0 mg/L 2,4-D + 1.0 mg/L Kinetin (8.00%). Effect of 2,4-D and Kinetin on the callus induction percentage and days of callus initiation in TCP 2 (*C. longa*) were graphically presented in Fig. 4.16 and Fig. 4.17 respectively.

From the present study it was observed that 2,4-D was the best auxin on the induction of callus than 2,4-D and kinetin combination in TCP 2 (*C. longa*). The similar type of findings was reported by Prakash *et al.* (2004).

**Fig. 4.16.** Effect of 2, 4-D and Kinetin on the callus induction percentage in TCP 2 (*C. longa*)

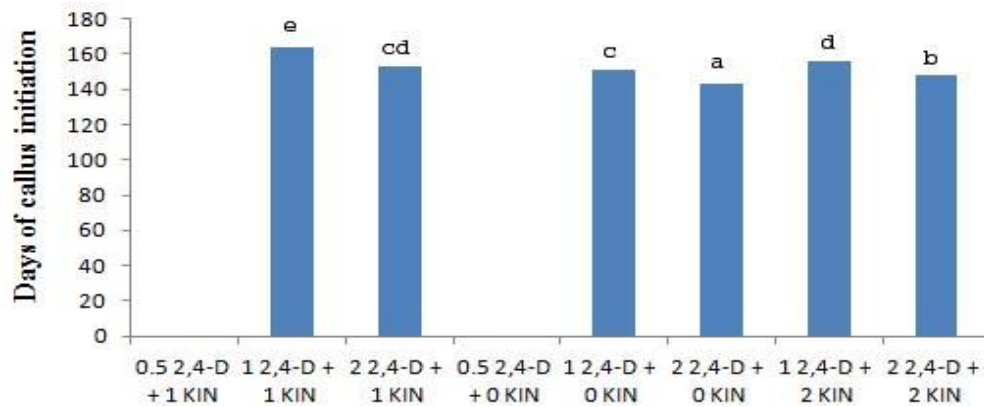


Fig. 4.17. Effect of 2, 4-D and Kinetin on days of callus initiation in TCP 2 (*C. longa*)

According to Sunitibala *et al.* (2001), callus cultures were established from shoot tips of *C. longa* in MS medium supplemented with 2.5 to 3.0 mg/L of 2,4-D. Regeneration of plantlets from callus was successfully conducted in MS medium supplemented with standard growth hormones and ultimately set roots.

But in our experiment the medium without hormone failed to induce callus even after 9 weeks. Induction of callus was better on MS medium supplemented with 2,4-D alone than 2,4-D and Kinetin combination. Callus was initiated through above tissue culture practices but failed to regenerate plantlets in spite of medium modifications of TCP 2 (*C. longa*).

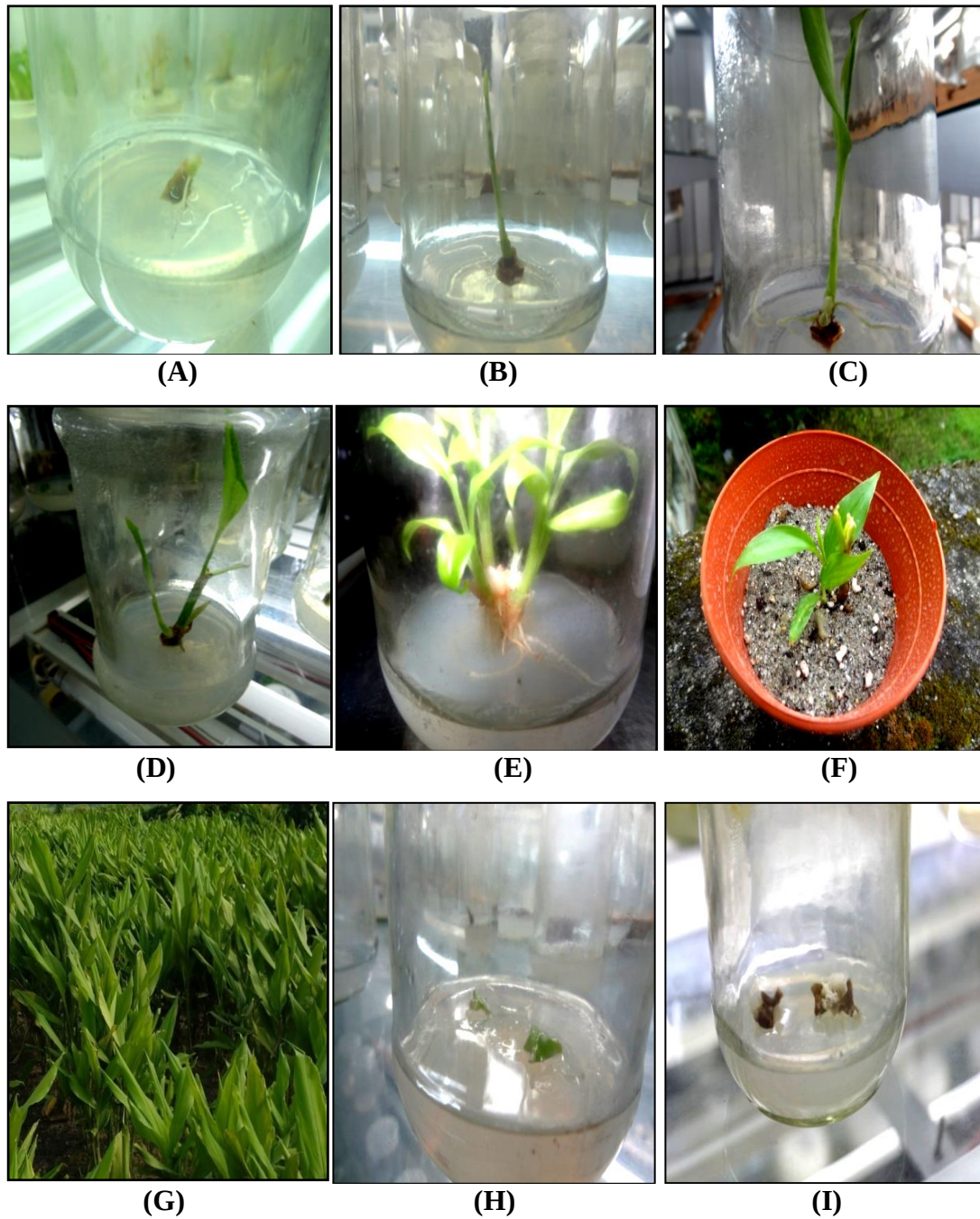


Plate 4.3. Different stages of *in vitro* micropropagation of TCP 2 (*C. longa*) (A) Shoot bud initiation after 14 days (without hormone), (B) Shoot initiation from bud after 35 days (without hormone), (C) Leaf initiation from shoot after 59 days (4.0 mg/L BAP + 2.0 mg/L NAA), (D) No. of shoots/explant (5.0 mg/L BAP + 2.0 mg/L NAA), (E) Root induction media (2.0 mg/L IBA, 82 days), (F) Hardening was done after 102 days (plastic pots with soilrite) (G) Developed plantlets transferred to the field after 136 days, (H) The induction of callus with leaf tissue after 70 days (2.0 mg/L 2,4-D) (I) Maximum callus growth after 220 days (2.0 mg/L 2,4-D)

4.4.2 Micropropagation of black turmeric (*Curcuma caesia* Roxb.)

4.4.2.1 Effect of growth regulators on shoot bud induction of black turmeric (*C. caesia*)

The present study was carried out to ascertain the *in vitro* efficacy of different hormones (BAP, Kinetin and IAA) on shoot bud induction of endangered medicinally important herb black turmeric (*C. caesia*). The basal medium used for shoot bud initiation purpose was MS media with different hormonal supplementations (Table 4.28).

Table 4.28: Effect of BAP, Kinetin and IAA on *in vitro* multiplication of shoot bud induction of black turmeric (*C. caesia*)

Hormone concentration (mg/L)			Days for multiple shoot initiation (mean* ± SE)	Shoot bud induction (%) (mean* ± SE)
BAP	Kinetin	IAA		
0.0	0.0	-	30.33 ± 1.080 ^g	32.67 ± 3.559 ^f
1.0	0.0	-	27.00 ± 0.707 ^{defg}	36.67 ± 2.160 ^f
1.0	0.5	-	26.67 ± 0.816 ^{def}	44.33 ± 2.483 ^e
2.0	0.0	-	26.00 ± 1.871 ^{cde}	46.67 ± 1.780 ^e
2.0	0.5	-	26.33 ± 1.780 ^{cde}	53.33 ± 1.633 ^{cd}
3.0	0.0	-	23.00 ± 1.225 ^{bc}	56.00 ± 1.871 ^{abcd}
3.0	0.5	-	21.33 ± 1.472 ^{ab}	52.67 ± 2.041 ^d
4.0	0.0	-	19.33 ± 0.408 ^a	59.00 ± 0.707 ^{ab}
4.0	0.5	-	20.33 ± 1.080 ^{ab}	57.67 ± 1.472 ^{abcd}
0.0	-	0.0	30.00 ± 0.707 ^{fg}	33.33 ± 2.273 ^f
0.5	-	0.25	27.00 ± 1.871 ^{defg}	35.33 ± 1.080 ^f
1.0	-	0.25	27.67 ± 1.080 ^{efg}	47.67 ± 1.472 ^e
1.5	-	0.5	23.67 ± 2.483 ^{bcd}	54.33 ± 2.483 ^{bcd}
2.0	-	0.75	18.00 ± 0.707 ^a	60.67 ± 0.816 ^a
2.0	-	1.0	19.33 ± 0.816 ^a	58.33 ± 1.780 ^{abc}

*Average of three replicates

The rhizome bud culture resulted that MS medium containing 2.0 mg/L BAP+ 0.75 mg/L IAA responded best followed by 4.0 mg/L BAP and 4.0 mg/L BAP + 0.5 mg/L kinetin. The shoot buds initiated in these treatments within 18 days, 19.33 days and 20 days respectively. On the other hand, moderate response was found with the medium containing the treatments 1.0 mg/L BAP + 0.5 mg/L kinetin, 2.0 mg/L BAP, 2.0 mg/L BAP + 0.5 mg/L kinetin, 3.0 mg/L BAP + 0.5 mg/L kinetin, 1.0 mg/L BAP + 0.25 mg/L IAA and 1.5 mg/L BAP + 0.5 mg/L IAA, whereas lowest was observed in full strength MS medium without any hormone.

No significant difference was found within these five different hormonal supplementations (3.0 mg/L BAP, 4.0 mg/L BAP, 4.0 mg/L BAP + 0.5 mg/L kinetin, 2.0 mg/L BAP + 0.75 mg/L IAA and 2.0 mg/L BAP + 1.0 mg/L IAA) because these hormonal combination grouped into the same subset (subset a). Maximum number of multiple shoot induction was observed after 8 weeks of culture. From the table, it was evident that out of fifteen different combinations, rhizome buds showed best performance in medium containing 2.0 mg/L BAP + 0.75 mg/L IAA in terms of shoot bud induction percentage (60.67). Effect of BAP, Kinetin and IAA on days for multiple shoot initiation and shoot bud induction percentage in black turmeric (*C. caesia*) was graphically presented in Fig. 4.18 and Fig. 4.19 respectively.

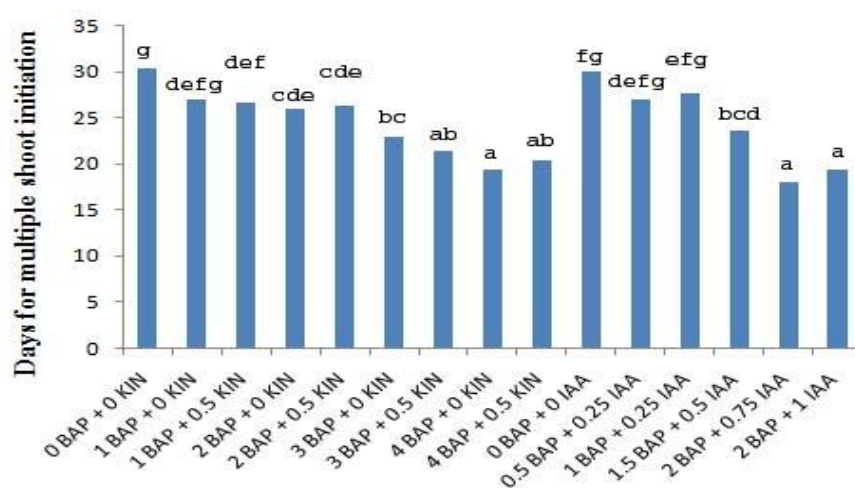


Fig. 4.18. Effect of BAP, Kinetin and IAA on days for multiple shoot initiation in black turmeric (*C. caesia*)

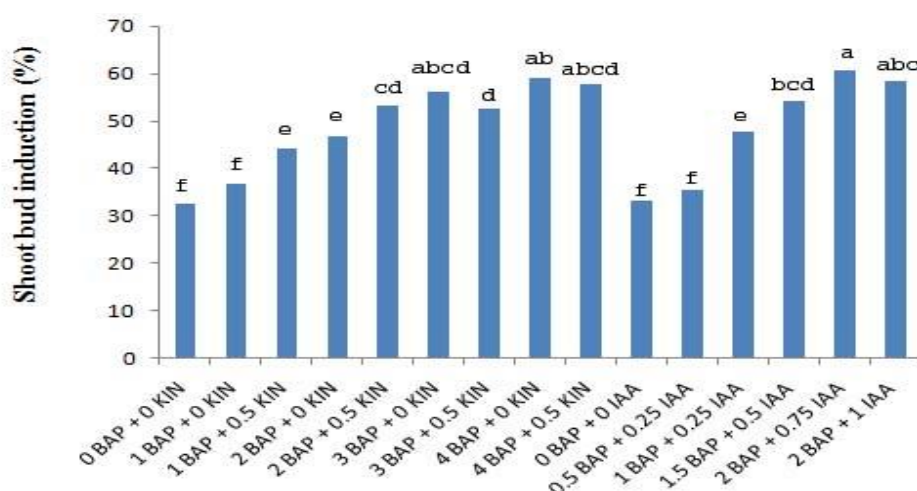


Fig. 4.19. Effect of BAP, Kinetin and IAA on shoot bud induction percentage in black turmeric (*C. caesia*)

In the present investigation, it was observed that with increase in the concentration of IAA above 0.75 mg/L, the rate of shoot multiplication declined. The similar type results were reported by Bharalee *et al.* (2005) and Shahinozzaman *et al.* (2013).

According to Shahinozzaman *et al.* (2013) the optimum shoot proliferation was found in case of black turmeric from MS medium containing 3.0 μ M BA + 0.5 μ M NAA and produced 10.38 shoots with 4.53 cm length after 8 weeks of culture. So the results of this study are in agreement with the works done earlier.

4.4.2.2 Effect of different growth regulators on multiple shoot regeneration and leaf emergence of black turmeric (*C. caesia*)

After initiation of shoot, the media composition was changed for multiple shoot proliferation. The basal medium was MS media with different hormonal supplementations as summarized in Table 4.29.

Table 4.29: Effect of BAP, Kinetin and IAA on *in vitro* multiplication of number of shoots/explants and number of leaves/shoot of black turmeric (*C. caesia*)

Hormone concentration (mg/L)			No. of shoots/ explant (mean* $\pm$ SE)	No. of leaves/ shoot (mean* $\pm$ SE)
BAP	Kinetin	IAA		
0.0	0.0	-	2.00 $\pm$ 1.225 ^{bc}	1.67 $\pm$ 0.816 ^c
1.0	0.0	-	1.67 $\pm$ 0.408 ^c	2.00 $\pm$ 0.000 ^{bc}
1.0	0.5	-	2.00 $\pm$ 0.000 ^{bc}	2.67 $\pm$ 0.816 ^{abc}
2.0	0.0	-	2.33 $\pm$ 0.408 ^{abc}	2.67 $\pm$ 0.408 ^{abc}
2.0	0.5	-	2.33 $\pm$ 0.408 ^{abc}	2.00 $\pm$ 0.000 ^{bc}
3.0	0.0	-	2.67 $\pm$ 1.080 ^{abc}	2.67 $\pm$ 0.408 ^{abc}
3.0	0.5	-	2.67 $\pm$ 0.408 ^{abc}	3.33 $\pm$ 0.408 ^{ab}
4.0	0.0	-	3.00 $\pm$ 0.707 ^{abc}	2.33 $\pm$ 0.408 ^{bc}
4.0	0.5	-	3.67 $\pm$ 0.408 ^{ab}	2.67 $\pm$ 1.080 ^{abc}
0.0	-	0.0	1.67 $\pm$ 0.816 ^c	1.33 $\pm$ 0.408 ^c
0.5	-	0.25	2.00 $\pm$ 0.000 ^{bc}	1.67 $\pm$ 0.816 ^c
1.0	-	0.25	2.67 $\pm$ 0.408 ^{abc}	2.00 $\pm$ 0.707 ^{bc}
1.5	-	0.5	2.67 $\pm$ 0.816 ^{abc}	4.00 $\pm$ 0.000 ^a
2.0	-	0.75	3.33 $\pm$ 0.408 ^{abc}	2.67 $\pm$ 0.408 ^{abc}
2.0	-	1.0	4.00 $\pm$ 0.707 ^a	3.33 $\pm$ 0.408 ^{ab}

*Average of three replicates

The rhizome buds culture in MS medium showed that MS medium supplemented with 2.0 mg/L BAP + 1.0 mg/L IAA showed highest shoot multiplication (4.00 shoots/explant) followed by 4.0 mg/L BAP + 0.5 mg/L kinetin

within 8 weeks of culture (3.67 shoots/explant) whereas lowest was found in 1.0 mg/L BAP containing medium and full strength MS medium without hormone (1.67 shoots/explant). A moderate response was found in 2.0 mg/L BAP, 2.0 mg/L BAP + 0.5 mg/L kinetin, 3.0 mg/L BAP, 3.0 mg/L BAP + 0.5 mg/L kinetin, 4.0 mg/L BAP, 1.0 mg/L BAP + 0.25 mg/L IAA and 1.5 mg/L BAP + 0.5 mg/L IAA containing medium.

There were no significant difference among the different treatments except 0.5 mg/L BAP + 0.25 mg/L IAA, 1.0 mg/L BAP + 0.5 mg/L Kinetin, 1.0 mg/L BAP and full strength MS medium without hormone because almost all the treatments came into the same subset (subset c). With an increase in the concentration of BAP and IAA, the number of shoots per explant increased. Out of fifteen different treatments, rhizome buds showed best performance in 2.0 mg/L BAP + 1.0 mg/L IAA containing medium in terms of number of shoots/explant. The effect of BAP, Kinetin and IAA on number of shoots/explant in black turmeric (*C. caesia*) has been graphically presented in Fig. 4.20.

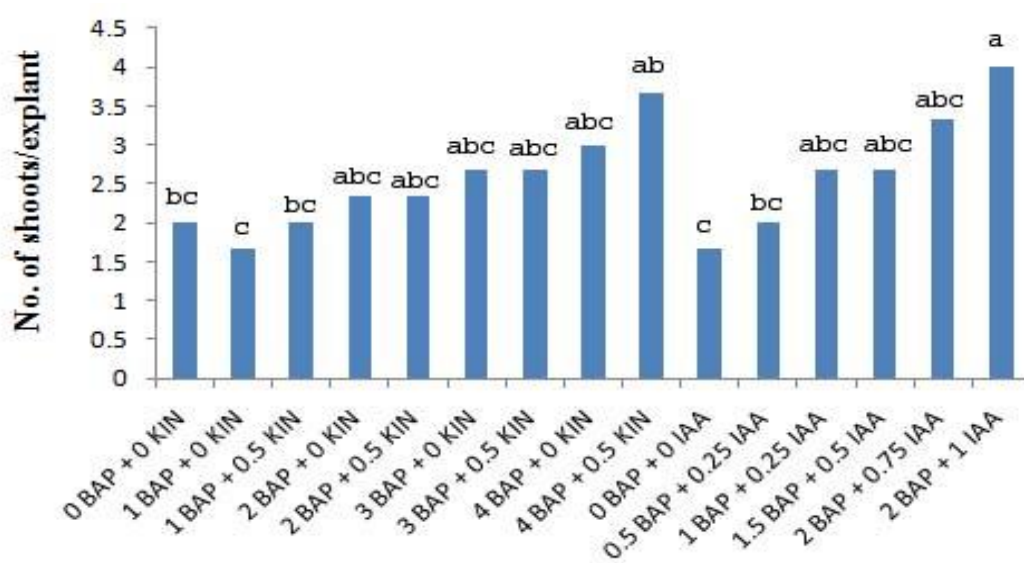


Fig. 4.20. Effect of BAP, Kinetin and IAA on number of shoots/explant in black turmeric (*C. caesia*)

The rhizome bud culture in MS medium in terms of number of leaves/shoot exhibited that MS medium with 1.5 mg/L BAP + 0.5 mg/L IAA responded best (4.00 leaves/shoot) followed by 2.0 mg/L BAP + 1.0 mg/L IAA and 3.0 mg/L BAP + 0.5 mg/L kinetin (3.33 leaves/shoot) whereas lowest was observed in full strength MS medium without hormone (1.33 leaves/shoot). On the other hand, moderate response

was found in all these media compositions namely 1.0 mg/L BAP + 0.5 mg/L kinetin, 2.0 mg/L BAP, 3.0 mg/L BAP, 4.0 mg/L BAP, 4.0 mg/L BAP + 0.5 mg/L kinetin and 2.0 mg/L BAP + 0.75 mg/L IAA.

No significant difference was found among the different treatments except 1.0 mg/L BAP, 2.0 mg/L BAP + 0.5 mg/L Kinetin, 4.0 mg/L BAP, 0.5 mg/L BAP + 0.25 mg/L IAA, 1.0 mg/L BAP + 0.25 mg/L IAA and full strength MS medium without hormone because almost all the treatments grouped into the same subset (subset c). Out of fifteen different hormonal supplementations, rhizome buds exhibited best performance in medium containing 1.5 mg/L BAP + 0.5 mg/L IAA in terms of number of leaves/shoot. The effect of BAP, Kinetin and IAA on number of leaves/shoot in black turmeric (*C. caesia*) has been graphically presented in Fig. 4.21.

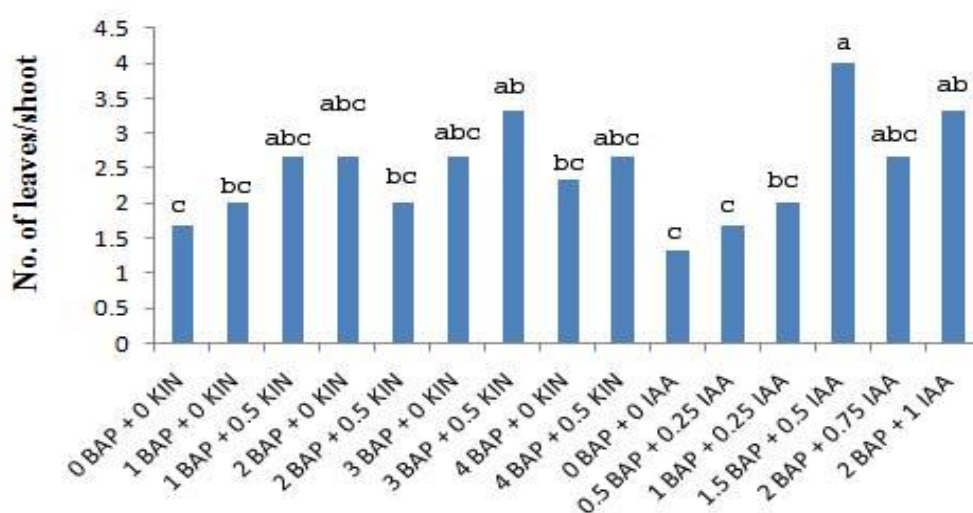


Fig. 4.21. Effect of BAP, Kinetin and IAA on number of leaves/shoot in black turmeric (*C. caesia*)

From the present study it was evident that with increase in the concentration of BAP alone, the rate of shoot multiplication increased. But, BAP and IAA combination showed better result than BAP alone. Analogous findings were reported by Balachandran *et al.* (1990) who observed that for shoot multiplication of *C. caesia* 3.0 mg/L BA supplemented with MS medium was optimum and Ghosh *et al.* (2013) who reported that MS medium supplemented with 2.5 mg/L of BAP + 1.5 mg/L NAA could produce 9.00 ± 0.57 shoots with an average height of 7.20 ± 1.01 cm.

4.4.2.3 Effect of different growth regulators on root initiation of black turmeric (*C. caesia*)

The well developed shoots were taken from shoot clumps and transferred to root induction medium i.e. full strength MS medium containing different hormonal combinations (Table 4.30). MS medium supplemented with a combination of 2.0 mg/L IBA gave the maximum number (11.67) of roots/shoot followed by 1.5 mg/L IBA (8.67 roots/shoot) while lowest number of roots/shoot (2.33) was found in full strength MS medium without hormone.

Table 4.30: Effect of BAP, Kinetin, IAA and IBA on *in vitro* multiplication of number of roots/shoot of black turmeric (*C. caesia*)

Hormone concentration (mg/L)				No. of roots/ shoot (mean* ± SE)
BAP	Kinetin	IAA	IBA	
0.0	0.0	-	0.0	2.67 ± 1.080 ^{fg}
0.0	0.0	-	0.5	3.67 ± 0.408 ^{efg}
0.0	0.5	-	0.5	4.67 ± 0.816 ^{def}
0.0	0.0	-	1.0	6.00 ± 0.707 ^{cd}
0.0	0.5	-	1.0	4.33 ± 1.633 ^{defg}
1.0	0.0	-	1.5	5.00 ± 0.000 ^{de}
0.0	0.5	-	1.5	4.00 ± 0.000 ^{defg}
0.0	0.0	-	2.0	8.33 ± 0.816 ^b
1.0	0.0	-	2.0	5.67 ± 0.408 ^{cde}
0.0	-	0.0	0.0	2.33 ± 1.080 ^g
0.0	-	0.0	1.0	6.00 ± 1.225 ^{cd}
1.0	-	0.0	1.0	5.00 ± 0.707 ^{de}
0.0	-	0.0	1.5	8.67 ± 0.408 ^b
0.0	-	0.5	1.5	7.33 ± 0.408 ^{bc}
0.0	-	0.0	2.0	11.67 ± 0.408 ^a

*Average of three replicates

Moderate response was found in 0.5 mg/L kinetin + 0.5 mg/L IBA, 1.0 mg/L IBA, 0.5 mg/L kinetin + 1.0 mg/L IBA, 1.0 mg/L BAP + 1.5 mg/L IBA, 1.0 mg/L BAP + 1.0 mg/L IBA and 1.0 mg/L BAP + 2.0 mg/L IBA containing medium. There was no significant difference within the three different hormonal supplementations (1.5 mg/L IBA, 0.5 mg/L IAA + 1.5 mg/L IBA and 2.0 mg/L IBA) because these hormonal combinations were appeared into same subset (subset b). Out of fifteen different hormonal supplementations, rhizome buds showed best performances in 2.0 mg/L IBA containing medium in terms of number of roots/shoot. Effect of BAP,

Kinetin, IAA and IBA on *in vitro* multiplication of number of roots/shoot of black turmeric (*C. caesia*) grown on MS medium was presented graphically in Fig. 4.22.

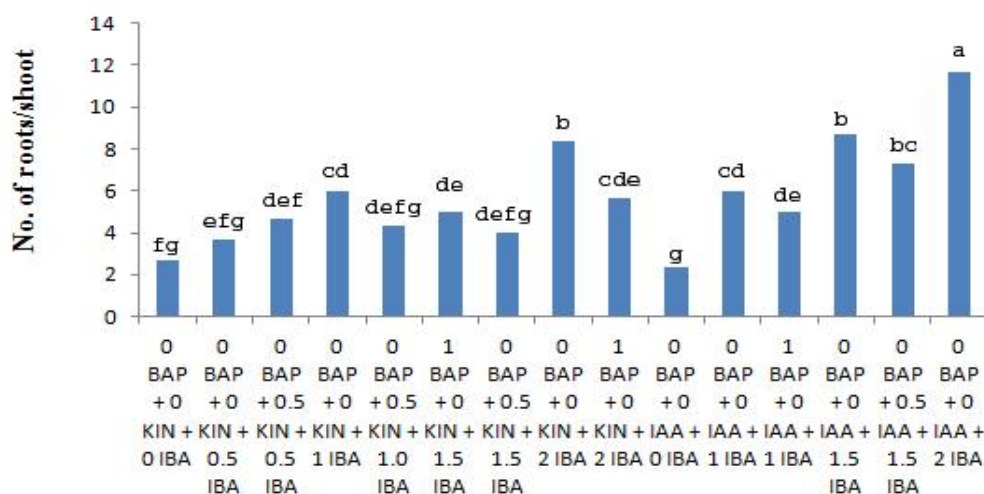


Fig. 4.22. Effect of BAP, Kinetin and IBA on number of roots/shoot in black turmeric (*C. caesia*)

Root initials were developed into a good root system within 14-18 days. Healthy rooted plantlets were transferred to the field after hardening. The survival rate of developed plantlets under field condition was 70%. The present study showed that shoot bud induction, number of shoots/explant, number of leaves/shoot and number of roots/shoot of black turmeric (*C. caesia*) was depended on the growth regulators used as supported by the observations of Singh *et al.* (2015).

It was observed in the present investigation that 2.0 mg/L IBA was optimum for the development of sufficient number of roots/shoot. Similar types of results were reported by Shahinozzaman *et al.* (2013) and Singh *et al.* (2015).

4.4.2.4 Effect of growth regulators on callus induction in black turmeric (*C. caesia*)

Callus induction in black turmeric (*C. caesia*) was achieved using different combinations of 2,4-D (0.5, 1.0 and 2.0 mg/L) along with Kinetin (1.0 and 2.0 mg/L) as noted in Table 4.31. Callus induction started after 3 weeks and maximum callus growth was obtained after 83.33 days in the medium supplemented with 2.0 mg/L of 2,4-D (31.33%) followed by 2.0 mg/L 2,4-D + 2.0 mg/L Kinetin (25.00%). Callus growth was relatively poor (14.67%) in the medium containing 1.0 mg/L 2,4-D + 1.0 mg/L Kinetin. The medium without hormone failed to induce callus even after 7

weeks of culture. Induction of callus was better on MS medium supplemented with 2,4-D alone than 2,4-D and Kinetin combination.

Table 4.31: Effect of 2, 4-D and Kinetin on the induction of callus in black turmeric (*C. caesia*)

Hormone concentration (mg/L)		Callus induction (%) (mean* $\pm$ SE)	Days of callus initiation (mean* $\pm$ SE)	Callus growth
2, 4- D	Kinetin			
0.5	1.0	0.00	-	-
1.0	1.0	14.67 $\pm$ 1.080 ^e	96.33 $\pm$ 1.633 ^d	+
2.0	1.0	16.33 $\pm$ 0.408 ^d	92.00 $\pm$ 1.414 ^{bc}	+
0.5	0.0	0.00	-	-
1.0	0.0	24.00 $\pm$ 0.707 ^b	90.67 $\pm$ 0.816 ^b	+++
2.0	0.0	31.33 $\pm$ 0.408 ^a	83.33 $\pm$ 0.408 ^a	++++
1.0	2.0	21.67 $\pm$ 0.816 ^c	91.67 $\pm$ 1.080 ^{bc}	++
2.0	2.0	25.00 $\pm$ 1.225 ^b	94.00 $\pm$ 0.707 ^c	+++

*Average of three replicates

Callus formation initiated through tissue culture practices but failed to regenerate plantlets even after modification of medium. The effect of 2,4-D and Kinetin on the callus induction percentage and days of callus initiation in black turmeric (*Curcuma caesia*) was graphically presented in Fig. 4.23 and Fig. 4.24 respectively.

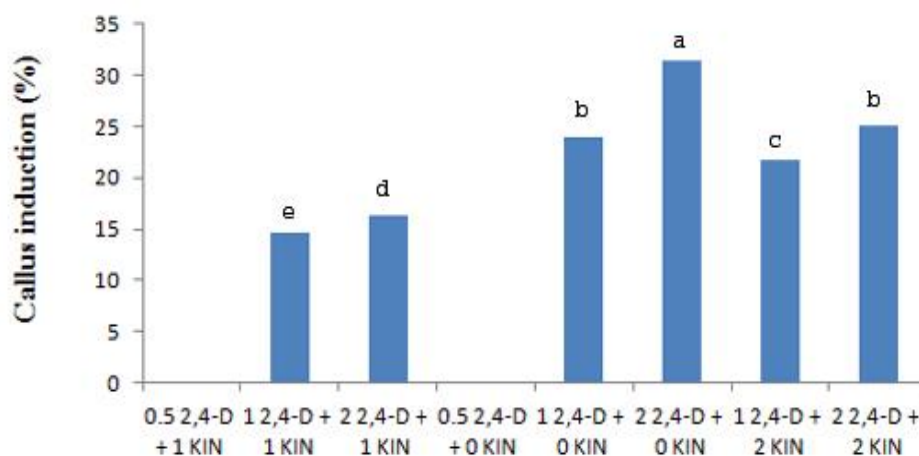


Fig. 4.23. Effect of 2, 4-D and Kinetin on the induction of callus in black turmeric (*C. caesia*)

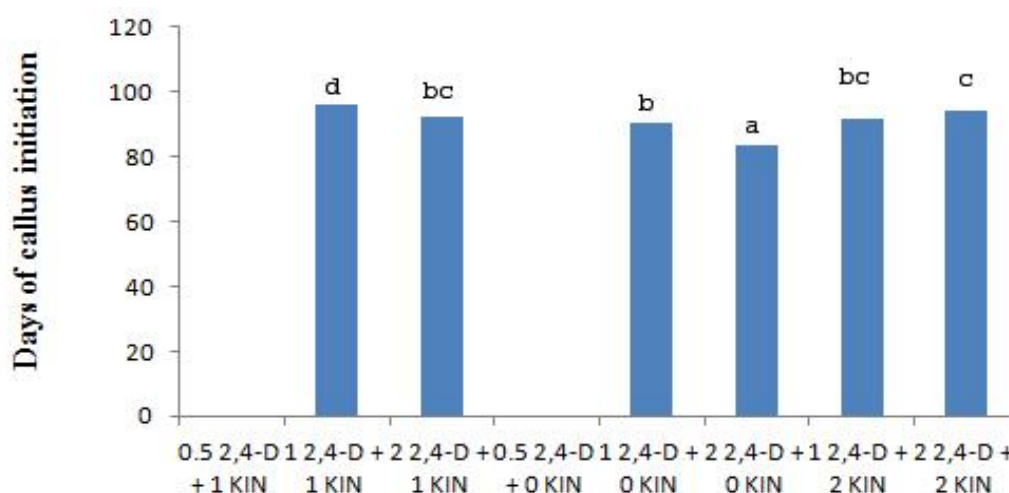


Fig. 4.24. Effect of 2, 4-D and Kinetin on days of callus initiation in black turmeric (*C. caesia*)

From the present study it was observed that 2,4-D was the best auxin on the induction of callus than combination of 2,4-D and kinetin in black turmeric (*C. caesia*). The similar type of findings was reported by Singh *et al.* (2015) and Md Zain (2013).

In vitro micropropagation of TCP 2 (*C. longa*) and black turmeric (*C. caesia*) was carried out with the help of different auxins and cytokinins (BAP, NAA, IAA, IBA, Kinetin and 2,4-D). For induction of multiple shooting of the rhizomes (TCP 2), a combination of BAP + NAA and BAP + Kinetin was used. BAP (6-Benzylaminopurine) is a first-generation synthetic cytokinin that is known for its role in plant growth and development responses in tissue culture by stimulating cell division (Zuraida, 2013). NAA is a synthetic auxin used for root initiation in the micro propagation techniques (Ali *et al.*, 2004). A higher concentration of BAP and lower dose of NAA help shown promising results in shoot induction in case of turmeric rhizomes (TCP 2) in our studies and in earlier studies (Ikeda and Tambe, 1989). In case of root induction only IBA was provided to the cultures. IBA is a plant hormone in the auxin family that is known for its role in root formation *in vitro* (Ghosh *et al.*, 2013). The dose of 2 ppm IBA supplemented in MS media has given the best root induction in the rhizome cultures. In case of *C. caesia* the combination of BAP + IAA was found better than BAP + Kinetin in multiple shooting.

In case of callus cultures combination of 2,4-D and Kinetin was tested. 2,4-D is a synthetic auxin and has prominent role in dedifferentiation of the plant tissues to induce callus (Prakash *et al.*, 2004). Kinetin is a type of cytokinin that promotes regeneration of plantlets from callus. 2 ppm 2,4-D without any kinetin was found to be the best in inducing callus. Even after application of kinetin in increasing concentration absolutely no regeneration of root initiation was notice in the calluses derived from turmeric leaves.

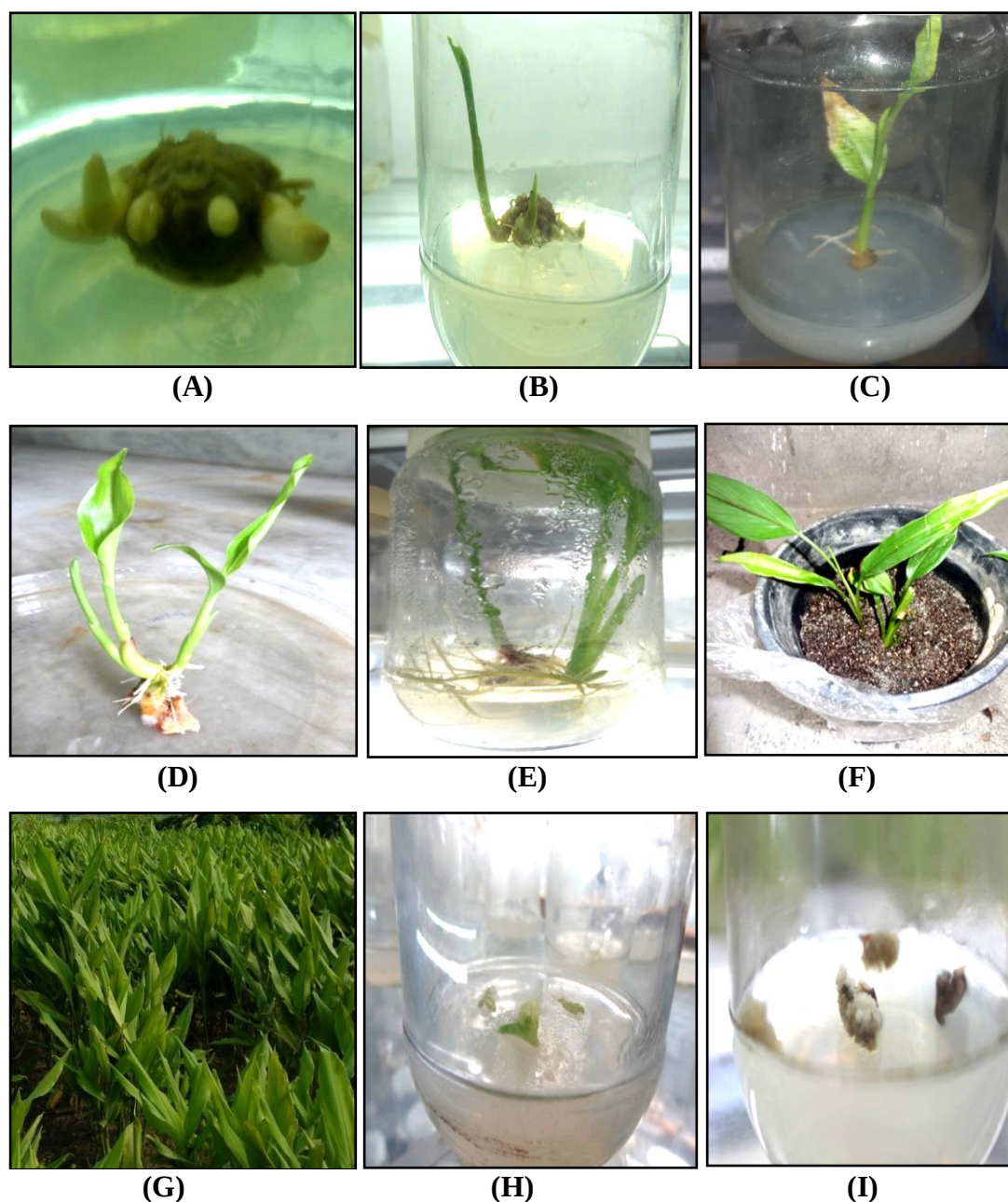


Plate 4.4. Different stages of *in vitro* micropropagation of black turmeric (*C. caesia*) (A) Shoot bud initiation after 15 days (without hormone), (B) Shoot initiation from bud after 32 days (without hormone) (C) Leaf initiation from shoot after 54 days (1.5 mg/L BAP + 0.5 mg/L IAA) (D) No. of shoots/ explant (2.0 mg/L BAP + 1.0 mg/L IAA) (E) Root induction media (2.0 mg/L IBA, 72 days) (F) Hardening was done after 90 days (plastic pots with soilrite) (G) Developed plantlets transferred to the field after 120 days (H) The induction of callus with leaf tissue after 65 days (2.0 mg/L 2,4-D) (I) Maximum callus growth after 154 days (2.0 mg/L 2,4-D)

Summary and Conclusion

The present research programme entitled “Biochemical characterization of turmeric (*Curcuma longa* L.) germplasms and standardization of protocol for micropropagation of selected cultivars” was carried out with three objectives namely (i) Scoring of collected germplasms of turmeric based on statistical analysis of phenotypic and biochemical characters, (ii) Estimation of quality related biochemical parameters of some selected cultivars/varieties and attempt to find correlation among different parameters, and (iii) Standardization of protocol for micropropagation of best of the selected cultivars.

Under the first objective fortyfive genotypes of turmeric, collected from the depository of AICRP on Spices at UBKV, were selected from a total of sixty on the basis of curcumin content, categorized as Group I having low (0.10 - 1.00%), Group II having medium (1.01 – 2.50%) and Group III having high (2.51- 5.50%) curcumin and were grown in the field for three years (2015-16 to 2017-18). Data of a total of nine parameters including seven phenotypic characters namely plant height, number of tillers per plant, number of leaves per plant, leaf length, leaf breadth, rhizome weight and yield and two biochemical characters *i.e.* curcumin and iron content in the rhizomes were recorded and subsequently analyzed statistically for different variability studies. The data of all the studied parameters exhibited significantly wide variations.

Variability studies including GCV, PCV, ECV, estimates of heritability (h^2) and expected GAM percentage in respect of yield and yield determining traits fulfill the basic requirement for crop improvement programme.

From the statistical analysis, highest GCV (75.99) and PCV (76.56) values were observed for curcumin content whereas lowest was recorded for iron content, GCV (4.18) and PCV (5.70). High estimates of heritability were recorded for curcumin content (98.52) whereas value was low for iron content (53.76). GAM

percentage was found highest in curcumin content (155.38) while lowest value was observed in yield (45.29).

As revealed from the results, very little difference between GCV and PCV was found for curcumin content followed by iron content, leaf length, leaf breadth and plant height and PCV values were higher in all the cases than GCV values. It can, therefore, be concluded that these characters were slightly too moderately influenced by environmental factors.

High heritability found for different parameter suggested that large variation for phenotypic variance was imputed to genotypic variance, and for this reason, selection would be assembled on the basis of phenotypic coefficient of variation. The values of GCV, PCV, h^2 and GAM were highest for average curcumin content. So, it can be concluded that individual plant selection based on these characters can effectively bring about genetic improvement in turmeric.

While determining the genotypic and phenotypic correlation coefficients it was observed that the genotypic correlation coefficient values were higher in magnitude than those of phenotypic correlation coefficient in most of the cases. The results of this analysis suggest that with increase in plant height, leaf number and leaf breadth there happens to be an increase in the leaf length and yield. Plant height, in particular, showed highly significant positive correlation with rhizome yield.

It can, therefore, be concluded that tall plants having longer leaves will be a suitable plant type for higher rhizome yield. The highly significant positive correlation between curcumin content and iron content leads to the conclusion that with the increase in curcumin content in the varieties, iron content will also be increased.

From path coefficient analysis it was observed that both plant height and iron content exhibited significantly positive direct effect on curcumin content (0.847 and 0.465 respectively). Leaf length showed positive direct effect on plant height (0.677) but indirect effect on curcumin content (-0.458). Most other characters showed significantly negligible effect on curcumin content.

It can, therefore, be concluded that plant height is one vital phenotypic character on which selection of curcumin rich and high yielding genotypes can be

made. Since iron content exhibited positive effect on curcumin content, so it can also be conferred that curcumin rich genotypes would also be rich in iron.

Distribution of turmeric genotypes into clusters was done by Mahalanobis D^2 analysis which is a suitable technique to estimate the divergence present in the genotypes and also helpful in selection of genetically different genotype(s). The total number of genotypes was distributed into eight clusters (cluster I to VIII) in which cluster I comprised of highest number of genotypes (14) and contributed 31.11% to form the cluster. Cluster V contained second highest number of genotypes (11) followed by cluster VIII (8), cluster IV and VII (3) whereas lowest number of genotypes was observed for cluster II, III and VI (2). Their contribution percentages towards clustering were 24.44%, 17.78%, 6.67% and 4.44% respectively.

From this clustering pattern it can be concluded that there was no association of genotypes, i.e. there was significant amount of diversity among the genotypes and each cluster was distantly related with the other. Among the clusters, cluster V was more divergent because it contained nine medium curcumin containing genotypes and two high curcumin containing genotypes.

Inter and intra cluster distance analysis, an index of genetic diversity among clusters, showed that inter cluster average D^2 value was maximum ($D^2 = 16.07$) between cluster VI and cluster VII indicating the genotypes of cluster VI remained far away from those of cluster VII and were genetically diverse. In case of intra cluster average D^2 value lowest difference between cluster II and III suggested least genetic differences among the genotypes of these clusters.

From the cluster mean values for different characters it was observed that cluster VII contained highest cluster mean values for three characters namely plant height (151.24), curcumin percentage (4.79) and iron content (53.56) whereas Cluster V and VIII had lowest mean values for one character namely rhizome weight (452.62) and leaf length (66.73) respectively. These cluster mean values are utilized by the breeders to fulfill the objectives of breeding program.

In case of genetic divergence analysis iron content contributed maximum (64.14%) towards genetic divergence occupying first rank (635) towards divergence whereas leaf number (0.20%) and plant height (0.20%) contributed to the minimum.

The distinctive values of the studied characters across genotypes can lead to the conclusion that each genotype is distinguished individually.

Principal component analysis revealed that the four principal components (PCs) with eigenvalues near about one accounted for 72.72% of the variability among 45 genotypes. PC 1 accounted for 29.26% of the total variation whereas PC 4 accounted for 10.84% of the total variation. The phenotypic characters which contributed more positively to PC 1 included plant height (0.55), leaf length (0.50), curcumin percentage (0.48), iron content (0.35) and leaf breadth (0.26). From the variable factor map of first two principal components it was revealed that plant height was the most important morphological character for increasing the rhizome yield of turmeric.

So it can be concluded that the genotypes giving higher values of these characters may be given more emphasis and hence better selection process for further crop improvement programme as well as for varietal improvement of turmeric.

Under the second objective, estimation of quality related biochemical parameters of some selected cultivars/varieties and correlation analysis among different parameters were done with standard methods.

The first and most important biochemical parameter in turmeric quality assessment is curcumin content of the rhizome. Results of analysis showed that among the selected genotypes, TCP 2 contained the highest curcumin (5.12%) whereas lowest curcumin content was recorded in TCP 154 and TCP 138 (both containing 0.14% curcumin). A few other varieties under Group III also contained high amount of curcumin, *e.g.* TCP 195 (5.04%) and TCP 67 (4.89%) which have the potential to be cultivated as economically profitable variety.

Colour is one of the most important attributes in determining quality of turmeric rhizome. Based on the curcumin content the color of the rhizome varies from deep orange to light yellow. From the result of colour parameter analysis it is observed that with increase in curcumin content the a^* value also increases indicating colour of the rhizome to be deep orange to red. Conversely, low curcumin containing genotypes give high b^* value and impart towards light yellow colour.

Study revealed that most of the genotypes under Group III with high curcumin level possessed deep yellow to deep orange colour whereas those of Group II having medium range of curcumin demonstrated light yellow to deep yellow colour. The genotypes under Group I having low curcumin exhibited mainly light yellow colour.

Results of total phenol content analysis showed that a large number of the turmeric genotypes under Group II and III namely TCP 222, TCP 84, TCP 64, TCP 194, TCP 2, TCP 67 and TCP 195 contained high total phenol whereas those of Group I possessed low total phenol. Highest phenol was present in TCP 247 (9.96 mg GAE/g) of Group II.

Curcumin is phenolic in nature and contributes highly to the total phenolics level of turmeric rhizomes. The reason of this might be due to the fact that the two phenolic hydroxyl groups of curcumin are linked to different benzene rings and allow them work freely without hindrance (Borra *et al.*, 2013).

Results of total protein content estimation revealed that turmeric genotypes richer in curcumin content had also higher amounts of proteins and total phenol content. One possible reason of this might be the presence of larger quantity of enzymes, mostly proteins, involved in the synthesis of curcumin as well as other phenolic compounds that contribute to the total phenol pool. Another reason of this high protein and phenol content might be the interference of phenolic compounds during estimation of protein by Folin-Ciocalteu reagent and vice-versa. Further study may throw more light in this area.

Turmeric is a good reservoir of minerals like calcium, iron, potassium, manganese, copper, zinc and magnesium. The iron content of the selected genotypes was determined in this study and was found to possess to the extent of 46.79–55.09 mg/100 g. So our daily requirement of iron, one highly essential mineral for our body, can partially be supplemented to some extent through consumption of turmeric powder of varieties rich in iron.

It has been established from a number of studies that crude fats as well as essential oils, mixture of numerous compounds, present in turmeric rhizomes contribute substantially to the health benefits and disease prevention properties of turmeric. The results of the present study shows that rhizomes of turmeric varieties

poor in curcumin, *e.g.* TCP 100, TCP 221, TCP 87 and TCP 154 under Group I in the present study, are comparatively richer (to a maximum of 6.94% in the studied genotypes) in crude fats. So, not keeping aside, these varieties may be included in turmeric cultivation programme to extract the crude fats and high priced essential oils. In addition, development of economically viable oil extraction methods may also help improve livelihood of a number of people.

Studies on the antioxidant activity (measured by DPPH, ABTS, TAC and CUPRAC assay methods) and activity of three antioxidant enzymes (superoxide dismutase, catalase, and peroxidase) indicated that both of these increased with increase of curcumin content. It is well established that curcumin is a good antioxidant. Turmeric also contains considerable amounts of other phenols which contribute to their high free radical scavenging activity.

The findings of this study specify the importance of turmeric as a readily available source of antioxidants in order to protect the diseases like cancer, diabetes and dementia for which free radicals are considered one of the major contributing factors. The antioxidant activity of curcumin can be moderated through antioxidant enzymes such as superoxide dismutase, catalase and peroxidase. These antioxidant enzymes are the main line of defense against free radicals in animal and plant cells.

The effect of pH on the stability of curcumin was also studied. Curcumin is comparatively stable at acidic pH but it rapidly decomposes at pH above neutral. When pH was changed from acidic to alkaline, colour turned from light yellow to dark brick red. The result was also confirmed by scanning in different wavelengths by UV-VIS spectrophotometer. Hence, it is not acceptable as a coloring agent in aqueous solutions of pH greater than 7.00. To investigate the effect of pH on rhizome colour development, initial pH and final pH after 2 hour of stirring of rhizome were measured. Initial pH was found highest in TCP 244 (8.47) which was from the high curcumin containing group. On the other hand, lowest pH value was recorded in TCP 100 (6.75) which belongs to the low curcumin containing group. TCP 244 showed highest final pH (8.08) whereas lowest was found in TCP 138 (6.67).

To study the plausible binding of curcumin with iron in different pH, reaction of curcumin with iron salt (FeCl_3) at different pH was measured. FeCl_3 (10 ppm)

showed maximum absorbance itself at 210 nm (1.857). When FeCl_3 was reacted with standard curcumin, the result showed maximum absorbance at 431 nm (0.448). The pH was then adjusted at 4.47. So, it can be assumed that iron reacts with curcumin and forms complex molecule *i.e.* chelate form. When the pH was further increased from 5 to 11, the maximum absorbance was shifted and the subsequently the values got reduced. This is may be due to the binding of iron with curcumin and chelate formation.

Curcumin content was positively correlated with the amount of iron, total phenol, total protein, catalase, peroxidase, SOD, antioxidant activity and redness (a^*) value. In earlier studies lots of reports are there about the color of rhizome and their biological activity. From our AICRP centre we have collected turmeric genotypes with wide variation in rhizome colour. Here we have showed that variation in rhizome color content of turmeric genotypes (*Curcuma longa* L.) reveals association with chemical composition, iron content, antioxidant activity and pH homeostasis.

From the correlation analysis, it can be concluded that increase in curcumin content positively influences the amount of iron, total phenol and protein (due to interference of one on the other during estimation by FCR), activity of catalase, peroxidase and superoxide dismutase, total antioxidant activity and redness of the genotypes.

Principal component analysis demonstrated that three principal components (PCs) accounted for 80.47% of variability among 45 turmeric genotypes. The principal components PC 1, PC 2 and PC 3 accounted for 63.89%, 9.27% and 7.30% of the total variation respectively. From these results, it can, therefore, be concluded that PC 1 is the most important one among the three principal components because almost all parameters are strongly and positively contributed.

The results depicts that curcumin is a major component present in turmeric rhizome that directly affects the color of turmeric. The findings revealed that majority of the biochemical parameters were found to be present in TCP 195 in highest or close to the highest quantity. So, this genotype was biochemically the best among the 45 genotype studied. But considering the yield, rhizome colour and most importantly the curcumin content of the genotype TCP 2, over TCP 195 this

genotype may be recommended to the farmers for the commercial cultivation practices.

Since the curcumin content, biochemical and yield parameters have suggested TCP 2 as the most promising genotype for this region. So, in the third objective for the standardization of micropropagation protocol TCP 2 was selected. Further standardization of micropropagation techniques of *Curcuma caesia* was carried out to conserve and mass multiply this endangered species.

In case of *Curcuma longa*, rhizome buds exhibited best performance (60.33%) in terms of shoot bud induction in MS medium containing 5.0 mg/L BAP + 1.0 mg/L NAA within 20 days whereas medium containing 5.0 mg/L BAP and 2.0 mg/L NAA showed best performance (4.00) in terms of number of shoots/culture. Maximum number of leaves/shoot (4.33) was observed in medium having 4.0 mg/L BAP + 2.0 mg/L NAA.

Curcuma caesia rhizome buds showed best performance in 2.0 mg/L BAP + 0.75 mg/L IAA containing MS medium in terms of shoot bud induction percentage (60.67) within 18 days. MS medium with 2.0 mg/L BAP + 1.0 mg/L IAA (4.00) responded best in case of formation of number of shoots/explant (4.00) whereas maximum number of leaves/shoot (4.00) was found in medium containing 1.5 mg/L BAP + 0.5 mg/L IAA.

MS medium supplemented with a combination of 2.0 mg/L IBA gave the highest number of roots in both species while 2.0 mg/L 2,4-D showed the best result of callus induction for both explants. But regeneration of plantlets from the callus culture through medium modifications could not be done successfully.

Among the selected two species of *Curcuma*, *C. caesia* showed more positive response in tissue culture with comparatively lower concentration of media components.

From the study it can be inferred that shoot multiplication can be considered as the best suitable tissue culture practices for the micropropagation of *Curcuma* species.

It may also be noted that the rhizome size needed for micropropagation of turmeric is smaller than conventional cultivation method implying that requirement of rhizomes will be much less than rhizome propagation method.

So, it can be concluded that tissue culture is a better practice for rapid multiplication of stock plant material to produce true to the type progeny plants under controlled *in vitro* conditions which is economically more viable in agricultural aspect.

Future Scope of Research

In the present study, scoring of the genotypes has been done based on phenotypic and biochemical characters. But, there are limitations in the use of phenotypic characters in genotype characterization like less availability of descriptors and influence of environment on phenotypic characters. As an alternative, more authentic and reliable method of molecular characterization is being done to ascertain the genetic variation among some genotypes of a crop at DNA level by adopting RAPD and SSR marker analysis. This can be applied for scoring the turmeric genotypes.

A number of biochemical parameters were not included in the present study. Quantification of those can be done to get more information about turmeric constituents.

More accurate analysis of chemical constituents of turmeric can be done by using HPLC, GC-MS etc.

Chemical composition of crude fats and essential oils of turmeric can be analyzed by GC-MS which was not included in the present programme.

Bioactivity of turmeric derived curcuminoids and related metabolites, medicinal effects of essential oils and phenols in human body can also be studied.

In the present micropropagation programme, rhizomes of the explant were of a single size. Further study can be done with rhizomes of varying sizes and lowest size giving positive results can be established which will be economically more viable.

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