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Influence of environmental factors on turmeric (*Curcuma longa* L.): Novel strategies to augment curcuminoid production

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ABSTRACT

Turmeric (*Curcuma longa* L.) belonging to the family Zingiberaceae is known worldwide for its multipurpose use; in medicine, cosmetics, food flavour and textile industries. Several value-added products obtained from turmeric includes the turmeric powder, essential oil, oleoresin and curcuminoids. Curcuminoids are phenylpropanoid derivatives and belong to diarylheptanoid class of secondary metabolites. The curcuminoids of turmeric include curcumin, demethoxycurcumin and bis-demethoxycurcumin. Curcuminis most sought after worldwide due to its tremendous medicinal importance.The curcumin content varies among turmeric cultivars. Environmental factors and growing locations influence variation in transcript levels of key curcuminoid biosynthesis pathway genes in turmeric cultivars which ultimately lead to the alteration in curcuminoid content. In this context, proper identification of elite turmeric cultivars by use of molecular and biochemical markers is necessary. Similarly, high yielding turmeric cultivars which are being produced must be subjected to agroclimatic filed trials prior to their release, which will largely benefit the turmeric industry. Metabolic engineering of curcuminoid biosynthesis pathway in *E. coli* and *S. cerevisiae* further provide a promising approach to produce these compounds in greater scale. The artificial neural network model will also greatly help to increase the yield of curcumin.

1. Introduction

Plant secondary metabolites (PSMs) are the compounds that are not essential for plant metabolic processes but are vital for a plant to interact with the environment. The plants communicate with external stimuli by the help of these compounds. These compounds are accumulated in the plant through various biochemical processes (Pavarini *et al*., 2012). PSMs are synthesized in environmental stress conditions and aid the plants to tackle the stress. The developmental as well as physiological stage of a plant is crucial for secondary metabolite production. Besides, they protect the plants from harmful UV light and help in seed dispersal.

Turmeric (*Curcuma longa* L.) a member of family Zingiberaceae has been in use in traditional medicine and as edible dye for many centuries. Severalvalue-added products can be obtained from turmeric which includes the turmeric powder, essential oil (leaf and rhizome), oleoresin and curcuminoids. Curcuminoids of turmeric comprise the compounds curcumin, demethoxycurcumin and bisdemethoxycurcumin. European Pharmacopoeia considers turmeric rhizome as an official medicinal product. Because of high medicinal importance, the demand for curcumin is increasing and thus a major growth in curcumin market is expected in future. India enjoys a monopoly of turmeric supply in international market (Angles *et al*., 2011). Turmeric essential oil is extracted by steam or hydro distillation procedures from both leaves and rhizomes. The characteristic turmeric aroma is due to the compound Tumer one which is the major compound of the turmeric essential oil (Sacchetti *et al*., 2005). Turmeric oil possesses antioxidant paracitidal and larvicidal properties (Ali *et al*., 2015).

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2. Effect of environmental factors on turmeric phytoconstituents

Plants often encounter various abiotic stresses such as drought, salinity, light, and temperature. These abiotic stresses remarkably cause alterations in plant growth and metabolism There are reports suggesting that 50-70% of crop yield losses are due to these environmental stresses. For sustaining these stress conditions plants respond by altering their metabolic pathways and undergo genetic modifications (Dos reis *et al*., 2012). The environmental and agroclimatic factors largely influence the secondary metabolite composition in turmeric and are responsible for the variation in curcumin content among turmeric cultivars (Anandaraj *et al*., 2014). Garg *et al.* (1999) showed that, variation also existed in the essential oil and curcumin content of *C. longa* grown in North Indian Plains. They collected 27 turmeric accessions and found that the essential oil percentage varied from 0.16% to 1.94% while the curcumin content ranged from 0.61%-1.45%. According to Lee *et al*. (2014) the curcuminoids content are affected both by the geographical location as well species. Their results revealed significant differences in the curcumin content of same species grown at two different regions. Light is an important factor which affects the quality and productivity of turmeric (Srikrishnah and Sutharsan, 2015). UV-B radiation induces the synthesis of L- phenylalanine ammonia-lyase (*PAL*) and peroxidase (*POD*) enzymes (Lavola *et al*., 2000). *PAL* is an important enzyme for the curcuminoid synthesis as the starter substrates involved in curcuminoid biosynthesis are synthesized from phenylalanine (Katsuyama *et al*., 2009). The availability of light plays an important role in the production of phenolics and terpenes (Ingersoll *et al*., 2010). Padmapriya *et al*. (2016) reported an increase in curcumin and total phenol content in turmeric plants grown under shade conditons (25-30%). Hossainand Ishimine (2005) cultivated turmeric under three different soil types (dark-red, grey and red soil) and found that dark red soil favoured the turmeric resulting in higher curcumin content than other soil types. Furthermore, high levels of any one nutrient solely increase the curcumin content (Srivastava *et al*., 2013). The combination of all the organic and inorganic factors is essential for higher curcumin content.

3. Effect of environmental factors on curcuminoid gene expression

A wide range of abiotic and biotic factors regulates the secondary metabolite biosynthesis in plants (Liu *et al*., 2015). Phenylpropanoids are one of the most important groups of secondary metabolites and key enzyme involved in their synthesis is *PAL* enzyme. There are reports of *PAL* regulation at transcriptional level by abiotic factors and pathogens (Khan *et al*., 2004). Curcumin is a derivative of phenylpropanoid and belongs to diarylheptanoid class of secondary metabolites (Roughley and Whiting, 1973). These diarylheptanoids are synthesized by Type III polyketide synthases (PKSs) (Schroder, 1997). Initially it was proposed that the curcuminoids are synthesized by two Type III (PKSs) which include diketide-CoA synthase (*DCS*) and curcumin synthase 1 (*CURS1*) enzymes. *DCS* is involved in the catalytic conversion of feruloyl-CoA and malonyl-CoA to feruloyldiketide-CoA (Kita *et al*., 2008) while *CURSl* catalyzes the conversion of feruloyldiketide-CoA to curcumin. *DCS* and *CURS1* enzymes are also involved in the synthesis of bisdemethoxycurcumin (Katsuyama *et al*., 2009). Subsequently Katsuyama *et al*. (2009) also identified two other type III polyketide synthases (PKSs) that are involved in curcumin biosynthesis viz. *CURS2* and *CURS3*. They have reported that feruloyl-CoA acts as a starter substrate for *CURS2* and *p*-coumaroyl-CoA and feruloyl-CoA acts as a starter substrate for *CURS3*. *CURS2* plays an important role in curcumin and bisdemethoxycurcumin while the *CURS3* participates in synthesis of all the three curcuminoids (Katsuyama *et al*., 2009). The curcuminoid biosynthesis pathway can be divided into upstream and downstream categories. The upstream genes comprise of *PAL, C4H, 4CL, HCT, C3H* and *OMT* while the downstream genes include the *DCS, CURS1, CURS2* and *CURS3* (Deepa *et al*., 2017) (Fig 1).

Elizabeth *et al*. (2011) have reported the variation in curcumin content within turmeric cultivars. They opined difference in the expression levels of genes encoding key enzymes in curcuminoid biosynthesis pathway leads to the variation in curcumin content (Katsuyama *et al*., 2009). Lovdal *et al*. (2010) studied the effect of environmental parameters on the different flavonoid pathway genes in tomato. They used different combinations of nitrogen and light. Flavanone 3-hydroxylase (*F3H*), flavonol synthase (*FLS*) and chalcone synthase (*CHS2*) gene expression was higher at low nitrogen and high light intensity. Similarly, the transcript levels of *PAL5* and *PAL6* also peaked at low nitrogen and high light intensity which suggests that nitrogen is an important factor contributing to flavonoid pathway. Resmi and Soniya (2012) characterized two new Type III polyketide synthase (PKSs), CIPKS9 and CIPKS10. The sequence of CIPKS9 was similar with chalcone synthase and CIPKS10 showed sequence similarity with curcuminoid synthase. They have analyzed the tissue specific expression of these genes and found that the *CIPKS9* transcript accumulation was higher in shoot and rhizome and less in leaves while the *CIPKS10* expression was higher in leaf and low in rhizome. Sheeja *et al*. (2015) studied the *CURS* gene expression in rhizomes of two turmeric cultivars, one

contained high curcumin content (*C. longa*) and other contained low curcumin content (*C. aromatica*). They have reported the up-regulation of curcuminoid pathway genes in *C. longa* and identified two novel PKSs that showed higher transcript accumulation in *C. longa* as compared to *C. aromatica.*

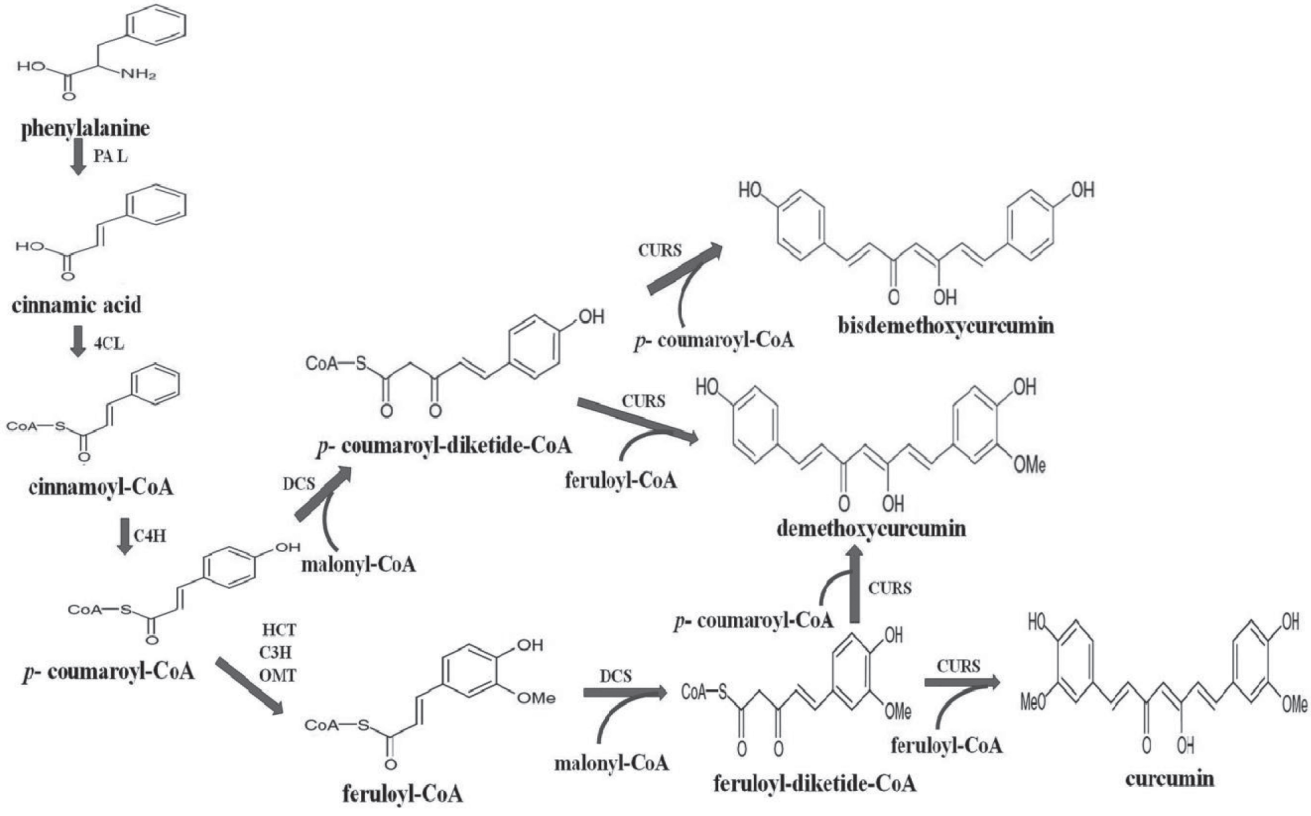
Li *et al*. (2015) studied the expression of curcuminoid biosynthetic pathway genes in four species (two wild and two cultivated). They have found that the expression of *CURS1* and *CURS2* genes was high in cultivated types while the *DCS* gene expression was comparatively low. But in wild types, *DCS* was highly expressed due to greater amount of p-coumaroyldiketide-CoA and they suggested that the difference in the expression could be due to difference in the availability of substrate concentration. According to Deepa *et al*. (2017) curcumin accumulation often exhibit spatio-temporal and environmental variation They studied the effect of environment on the *PKS* (*DCS, CURS1, CURS2* and *CURS3*) genes by selecting two turmeric genotypes with high curcumin (IISR Prathiba) and low curcumin content (Accession 449). The genotypes were planted at two different regions (Kozhikode and Coimbatore) and have found that the expression pattern was similar in both the genotypes where the plants at Kozhikode showed the highest expression and the plants at Coimbatore had the least expression, which clearly shows the impact of environmental factors and growing location on the curcuminoid biosynthetic genes.

Phenylalanine ammonia- lyase (*PAL*), 4-Coumarate CoA ligase (*4CL*), Cinnamate-4-hydroxylase (*C4H*), Hydroxycinnamoyltransferase (*HCT*), Cinnamate-3-hydroxylyase (*C3H*), O-methlytransferase (*OMT*), Diektide CoA synthase (*DCS*), Curcumin synthase (*CURS*).

4. Novel strategies to augment curcuminoids production

4.1. Molecular markers for identification elite genotypes

Analysis of morphological and phytochemical yield alone is not sufficient for elite genotype identification as the secondary metabolites are subject to variation under different environmental conditions. Though there are many high yielding turmeric cultivars, rigorous identification of the cultivars is essential because of their morphological resemblance (Sahoo *et al*., 2017). Biochemical markers were used to eliminate the duplicate turmeric varieties (Shamina *et al*., 1998). The assessment of turmeric genetic diversity is prerequisite for its breeding programme (Nass, 2001). The different molecular markers techniques which are used for elite genotypes identification in turmeric include random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) (Panda *et al*., 2007; Angel *et al*., 2008), Simple Sequence Repeat (SSR) (Sigrist *et al*., 2010; Joshi *et al*.,



2010; Sahoo *et al*., 2017) and Directed Amplification of Minisatellite DNA (Verma *et al*., 2015). Turmeric is propagatedvegetatively by means of rhizomes so hybridization is mostly unsuccessful. The elite genotypes are typically identified by field trails. Therefore, molecular markers will play a crucial role for germplasm identification and aid in boosting the turmeric quality.

In comparison to the biochemical markers the molecular markers are less influenced by the environment and thus will accurately identify the germplasm (Thimmappaiah *et al*., 2009; Cheng and Huang, 2009). RAPD and ISSR markers mostly widely used molecular markers for genetic diversity analysis (Ebrahimi *et al*., 2009). SSR markers are gaining much more importance than the RAPD and ISSR markers. The problem with RAPD markers is poor reproducibility of banding pattern (Mei *et al*., 2015). Besides, both the RAPD and ISSR markers are dominant and less reliable. The SSR markers offer numerous advantages than the other markers as they are co-dominant with high reproducibility and easy automation (UPOV/INF/17/1 2010 guideline). Moreover, SSRs are mostly two types: genomic SSRs and EST-SSRs. As compared to the genomic SSRs the EST-SSRs are more advantageous as they are derived from the expressed sequenced data and thus can be improve the applicability of genetic markers by expressing the variation in transcribed gene (Scott *et al*., 2000). Several studies have reported the use of SSR markers in cultivar identification and genotyping of different species (Koussao *et al*., 2014; Basheer-Salimia *et al*., 2014). Sahoo *et al*. (2017) have reported the use of EST-SSR marker for the identification and authentication of two high yielding turmeric cultivars (Lakadong and Suvarna). Thus, more studies are needed on this aspect which will enable proper identification of elite turmeric cultivars.

4.2. Evaluation of elite turmeric cultivars by planting at different agroclimatic zones

The demand for curcumin is increasing day-by-day (Li *et al*., 2015). Turmeric cultivars with high percentage of curcumin are attracting the worldwide market. However, variation in curcumin percentage in turmeric when grown at different places restricts the export potential. Though India is the largest producer of turmeric with many yielding turmeric cultivars, the average productivity is not satisfactory (Ayer, 2017). When a high curcumin yielding cultivar is cultivated in places other than its place of origin the curcumin percentage falls remarkably, thus affecting their commercial potential. The curcuminpercent in turmeric cultivars range from 2-7% (Sasikumar, 2005). Anandaraj *et al.* (2014) evaluated the curcumin content of eleven different turmeric cultivars across ten different locations in India for selection of stable genotypes with respect to different environmental parameters. The mean curcumin content of all the cultivars ranged from 4.22% -5.78%. The results from their study indicated that Mega Turmeric, IISR Kedaram and IISR Prathiba were highly stable and could be used in breeding programs for obtaining high dry yield and curcumin content. Significant differences in the morphological characters, essential oil and curcumin of high yielding turmeric cultivars (Surama and Roma) were observed when grown at different agroclimatic zones (Sandeep *et al*., 2016). The variation in the curcumin content was from 1.5-5% (Surama) and 1.4-5% (Roma). Soil nutrients (Nitrogen, Phosphorous and Potassium), Soil pH and altitude were most sensitive factors for curcumin content. Variation in secondary metabolite production with varying soil nutrients has also been reported in other plants (Alam and Naik, 2009; Ramakrishna and Ravishankar, 2011). Kandasamy *et al.* (2012) reported positive effect of potassium on curcumin yield. Gupta *et al*. (2015) have evaluated genetic divergence among 65 turmeric genotypes with respect to thirteen agro-morphological traits. They have grouped the genotypes into seven clusters and reported that the genotypes of cluster VI and cluster VII can be utilized for turmeric breeding. Thus, subjecting high yielding turmeric cultivars to different agroclimatic zones prior to their release will not only help in understanding the best environmental conditions for maximum phytochemical yield but also for managing the soil parameters for enhancing curcumin and essential oil.

4.3. Biotechnology approaches

Curcuminoids have enormous importance worldwide because of their wide range of medicinal properties and the research on curcumin has doubled since the past few years. Like all other secondary metabolites,the curcuminoids are also influenced by environmental factors and are accumulated over long period of time in plants. Besides, they are also subjected to seasonal variation (Fang *et al*., 2017). Furthermore, chemical synthesis of curcumin is costly (Rodrigues *et al*., 2015). To meet the demand of curcumin, there is need for novel strategies whereby the curcumin production can be enhanced. One such strategy that can be employed for enhancing curcuminoid content is heterologous production and metabolic engineering. Both microorganisms and plants can be used as for metabolic engineering. The heterologous production of curcuminoids in microrganisms is mostly preferred because larger amounts can be produced at low time (Lussier *et al*., 2012). The heterologous production in plants is costly and also several complications associated with the genetically modified plants in society. In addition the rules for genetically modified organisms are simpler than crops (Halls and Yu, 2008). Based, upon these facts the microorganisms are more preferred than plants for

the heterologous curcuminoid production. The use of appropriate synthetic enzymes are the key components for successful heterologous production of the desired compounds. The production of curcuminoids can be enhanced by utilization of substitute enzymes from other microorganisms that are well-suited with the heterologous host which permit greater curcuminoid yield. Likewise, more specific enzymes must be identified which will also improve the curcuminoid production with less preferred byproducts.

Katsuyama *et al*. (2008) for the first time reported the production of curcuminoids in *E. coli* by using tyrosine/ phenylalanine as the starter substrate, which is then converted to carboxylic acids by phenylalanine ammonia lyase (*PAL*). The carboxylic acids are converted to CoA esters by 4CL (4-coumarate-CoA ligase) and finally to curcuminoids by curcuminoids synthase (CUS). Acetyl-CoA carboxylase (ACC) was overexpressed for increasing the malonyl-CoA in *E. coli* as malonyl-CoA is also necessary for curcuminoid biosynthesis (Katsuyama *et al*., 2007). Generally the malonyl-CoA in microorganisms is utilized in fatty acid production which is the reason for low availability of malonyl-CoA for recombinant pathways and there are reports whereby ACC overexpression resulted in increase of bisdemethoxycurcumin (BDMC) titers (Xu *et al*., 2011). Till date much of the work on heterologous curcuminoid production is done using *E. coli* as host (Rodrigues *et al*., 2015). Engineering of curcuminoids using *S. cerevisiae* in comparison to *E. coli* offers several advantages as later is an eukaryotic organism and posseses post translation machinery. Besides, the cellular compartment of yeast is also similar to that of plant cell (Jiang *et al*., 2005). In curcuminoid biosynthesis pathway cinnamate-4-hydroxylase (*C4H*) is an important enzyme which converts cinnamic acid tocoumaric acid. In case of *E. coli*expression of *C4H* is challenging but in yeast there is no such problem. Several studies demonstrated the successful cloning and expression of *C4H* in *S. cerevisiae* from different plants (Yan *et al*., 2005; Trantas *et al*., 2009; Shin *et al*., 2012). Fang *et al*. (2017) engineered curcuminoid pathway in *E. coli*and have reported a co-culture technique for rapid production of curcuminoids from glucose. They have used two different strains of *E. coli* for conversion of glucose substrate to curcuminoid and have found out that co-culture led to the greater curcuminoid production than the single *E. coli* strain.

4.4. Artificial neural network (ANN) for predicting the curcumin content

The artificial neural network (ANN) is a mathematical model based upon statistical algorithms which resemble the mammalian neural network. The model consists of three layers of design: input layer in which data is fed, hidden layer in which the data processing occurs and outer layer which generates the result. Because of their high efficiency and accuracy for large data sets, the ANNs have wide range of applications from marketing, industry, finance, medicine and agriculture. ANN was used for predicting the yield of wheat in Argentine Pampas region (Alvarez, 2009). Artificial neural network models were developed for increasing the podophyllotoxin (anticancer compound) yield in *Podophyllyumhexandrum* (Alam and Naik, 2009). Similarly, the yield of sunflower in response to salinity and soil moisture was predicted by ANN model (Dai *et al*., 2011). Akbar *et al*. (2016) developed a prediction model based upon ANN for site specific cultivation of turmeric for maximum curcumin production. Thus, artificial neural network will help to generate high yielding turmeric varieties which will perform consistently across different agroclimatic zones.

5. Future Perspectives

The demand for quality turmeric products world over has triggered the production of this species many folds worldwide. There is an increase in demand for curcumin day by day. Moreover, the demand for rhizome and leaf essential oil is also increasing in the global market. In this article an overview of the impact of environmental factors on secondary metabolite contents in *Curcuma longa* L. is presented. In this context, the identification of elite turmeric varieties and their evaluation by planting at different agroclimatic zones is extremely important. In addition, expression of genes responsible for curcumin production in different environment and soil bears immense potential for future research and development. However, there are several critical factors which need to be addressed in research pertaining to improvement in turmeric production. In addition, more studies on heterologous production and metabolic engineering by employing new hosts are needed which will increase the curcumin production to a greater extent.

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