

**Assessment of Turmeric (*Curcuma longa* L.) Varieties for
Yield and Curcumin Content**

by

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Abstract

Turmeric (*Curcuma longa* L.) is a rhizomatous herbaceous perennial plant belonging to the ginger family, *Zingiberaceae*. Currently, more than 80% of turmeric is produced by India and turmeric products are exported to numerous countries. Other Asian countries, including China, Vietnam, Pakistan and Japan also grow significant amounts of turmeric. With the development of medicinal related research, turmeric shows huge potential impacts on cure cancer, prevent Alzheimer's disease and treat other diseases caused by inflammation. Turmeric is a new crop in Alabama. There is little available published information related to cultivation and planting varieties of turmeric in the United States, however turmeric has been successfully grown on the Auburn University Agronomy Farm since 2006. Researchers and farmers lack information on turmeric varieties that produce high yield and high content of curcumin, which determine the final benefits from this crop. Turmeric varieties were collected from various sources and tested in field trials during 2016 through 2018. Turmeric rhizomes were planted in pots in greenhouse and the sprouted plants were transplanted to field. Published cultivation practices were applied, including irrigation, fertilization and pest control. We harvested the turmeric rhizomes, collected data and made selections based on rhizome yield and curcumin concentration and curcumin

yield. A new variety was added in 2017. Four varieties were selected for the last field test in 2018. Finally, recommendations were made as to which turmeric varieties were best based upon intended uses or objectives, such as, high rhizome yield, high curcumin concentration and high curcumin yield.

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List of Abbreviations

ai	Stability Parameter
BA	N-(Phenylmethyl)-9H-purin-6-amine
B/C	Benefit cost ratio
CV	Coefficient of Variation
DM	Diferuloylmethane
DMC	Desmethoxycurcumin
BDMC	Bisdemethoxycurcumin
EMS	Ethylmethylsulfone
FYM	Manure
GA ₃	Gibberellic acid
GNPs	β -D-glucan nanoparticles
HPLC	High-performance liquid chromatography
HSC	High Stability Coefficient
IW/CPE	Irrigation water/cumulative pan evaporation
LAI	Leaf area index
NAA	1-Na-phthaleneacetic acid
OMRI	Organic Materials Review Institute

S Standard Deviation

USFDA The United States Food and Drug Administrat

Chapter 1 Literature Review

Turmeric (*Curcuma longa* L.) is a rhizomatous herbaceous perennial plant belonging to the ginger family, *Zingiberaceae*. Currently, more than 80% of turmeric is produced by India and turmeric products are sent to numerous countries. China also exports turmeric to supply this demand (Wickenberg et al., 2010), but India still is the biggest consumer, producer and exporter of turmeric over the world. India planted almost 173 thousand hectares of turmeric during the year 2005-2006 (Bansal et al., 2008). During the ancient India, turmeric was regarded as a gift from nature, this magic plant provided people food and health. Now with the development of modern chemical analysis methods, turmeric offers potential uses in new areas, like pharmacy.

Botanical Description

The genus *Curcuma*, one of the most important members of *Zingiberaceae* family, is composed of approximately 110 species, many of which have been shown to have various uses (Sasikumar, 2005). It is common in tropical Asia and the Asia-Pacific areas. The genus name originated from the Sanskrit 'kuṅkuma' (Sasikumar, 2005). *Curcuma longa* (L.) is the most important and famous species, generally known as turmeric. Around four or five months after planting turmeric starts flowering. Turmeric flowers need 7 to 11 days to reach blossoming stage and blossoming normally continues another 7 to 11 days (Patnaik et al., 1960). For *C. longa*, the flowering season is from September to December,

specific flowering duration is from 118 to 143 days after planting (Nambiar et al., 1982).

Turmeric is a highly self-pollinating crop, a few varieties cross breed and can produce seeds, but the crop is mainly reproduced asexually by planting rhizomes (Jayaprakasha et al., 2001). Most of varieties of turmeric have been cultivated separately in different places. The fleshy, branched, interlaced rhizome has primary and secondary rhizomes, and the shoots mostly come from the mother rhizomes (Bansal et al., 2008). Its tuber always shows aromatic property with yellow to brown color and 5 to 8 cm long, 1.5 cm thick, generally color of inside part is deeper. The primary rhizome is called as “mother rhizome” or bulb, pear-shaped in the center. The branches of mother rhizomes are the secondary rhizomes, called “finger rhizomes”. Just like ginger rhizome, turmeric finger rhizome only germinates on a main axis. The shape of the rhizome is conical, hemispherical and cylindrical (Jayaprakasha et al., 2001). Turmeric has special cincinnus inflorescence, in which the successive pedicels are arranged in a sort of spiral. But turmeric usually cannot breed real fruit. In fact, turmeric propagates by its rhizomes. Both mother rhizomes and finger rhizomes may be used as seed rhizomes, but finger rhizomes are more commonly used in commercial production than are mother rhizomes. The top of mother rhizome develops the above-ground parts, i.e. shoots and leaves. Finger rhizomes sometime enlarge and form branches. The first-order branches are known as primary fingers; new branches develop from them and are called secondary fingers; and then there are tertiary fingers. Some nodes on primary fingers are the future secondary fingers.

Besides the mother rhizome, some fingers also can turn into mother rhizomes and develop shoots. Others show positive geotropic growth with all secondary and tertiary fingers. The green leaf stems are nearly 1 m tall, and leaf blade length usually ranges from 37 to 38 cm with a maximum of 50 cm. The petiole is narrow but widens at the base of the blade (Maheshwari et al., 2006).

Since the 1930s, T. Suguira (1936) carried out primal chromosome research on dicotyledonous plants, including Zingiberaceae, over a period of around 10 years. This is the first available report of cytological studies on turmeric. Turmeric materials were collected from a Japanese island called “Luchuan”. Suguira (1936) concluded that *Curcuma longa* is diploid with 64 chromosomes and published figures of turmeric chromosomes, clearly showing short rod-like chromosomes. Similar research conducted later on related species showed that *C. amada* and *C. aromatica* have 42 chromosomes, while *C. zedoaria* and *C. petiolate* both have 64 chromosomes (Venkatasubban, 1943; Nair and Sasikumar, 2009).

Other researchers found that turmeric varieties could have different numbers of chromosomes, ranging from 62 to 64 (Ramachandran, 1969). And based on already known acknowledge, Ramachandran speculated that triploid *C. longa* may be a hybrid between two other species in Zingiberaceae, *C. aromatica* and diploid *C. longa*.

The process of growth and development of turmeric may be separated into three stages. The first two months are the sprouting stage or could be called the growth of seed

rhizome stage. During the next approximately one month, finger rhizomes appear and the turmeric plant has accumulated abundant nutrient and energy to develop the shoots and leaves. After that the whole plant has finished all preparation and then gives the priority to the growth of rhizome. Four or five months after planting, turmeric could reach the highest LAI (leaf area index). Under shade, the highest LAI may occur one month later. The highest LAI for a turmeric variety under these two conditions, i.e. open and shade, could be same or differ significantly, depending on the variety (Padmapriya et al., 2016).

Li et al. (1997) separated turmeric growth into two stages. The first stage is from rhizome to leaves. At the maximum point, more than 75% dry matter is in leaves. Then the growth center gradually moves to the rhizomes. Finally, more than 40% dry matter will be saved in rhizomes (Li et al., 1997).

History

The etymology of turmeric is obscure. The first given name of turmeric in Europe was *terramerita* as a neo-Latin form (Guthrie, 2015). Then Europe started to import turmeric powder from India, the homeland of turmeric. Turmeric's original name in French was *terre merite*. Both two forms could be translated as meritorious earth or deserving earth, but these are just our guess. With the development of turmeric trade, turmeric got a new name, *safran des Indes*, which was common in France until nineteenth century. This name is derived from saffron, which is another plant, but in the history, for a long time, when Indian people mentioned saffron, just meant turmeric. The name,

“Termeryte,” first appeared in English. It is close to the universal name we know, turmeric. The strange point is the different final consonant. Finally, sometime in 1500s, turmeric occurred and regarded as the universal name (Guthrie, 2015).

In Sanskrit language turmeric has many synonyms. The interesting thing is that these names relate to different qualities. Like a variety called “Pavitra”, it is for blessing in Sanskrit. Pavitra just means holy. Similarly, in Chinese, turmeric is called “Chiang Huang”. Chiang is the name of ginger, a relative of turmeric, huang means yellow. In other word, Chinese defined a plant which looks like ginger with yellow rhizome, so turmeric has its Chinese name, chiang huang (Mory, 2016).

Turmeric has been grown at least 2500 years in some Asian countries, especially in India and Indonesia, and it later appeared in Africa and Caribbean. However, European and American herbalists had little interests in turmeric until the 20th century (Bansal et al., 2008). It was estimated that turmeric had been cultivated 4000 to 6000 years ago but documents to prove it are lacking. It can be confirmed that Marco Polo (1280 AD) referred to turmeric as Indian saffron, which was used for dying cloths and it was mentioned in the Pent-Sao of the 7th century in China (Bansal et al., 2008). At first, turmeric was likely used for yellow dye, then humans found turmeric can make foods yellow and orange and spice foods. Ancient scientists believed that turmeric can protect humans from some diseases, and then turmeric was added into therapy as medicine. The primary coloring substance of turmeric’s rhizomatous tissues was extracted and named

curcumin nearly 200 years ago (Bansal et al., 2008). Now we believe that initial turmeric varieties are selected from old wild turmeric (*Curcuma aromatica*) of India, Sri Lanka and the eastern Himalayas (Yadav et al., 2013).

Some inventions opened a door to cosmetics. Components of turmeric like curcumin can be combined with glycolic acid to create makeup.

Turmeric's Utilization in Food Production

Turmeric powder is warm, bitter and earthy like pepper and mustard, and looks just like curry which is one of the most popular spice in many southern Asian countries.

Turmeric can be used by itself or combined with other spices. Traditionally turmeric is used in various cuisines giving flavors as well as a coloring agent for curry foods. Curry powder is made mainly from dried turmeric powder. Pure turmeric powder had the highest curcumin concentration compared to other substances made from turmeric like curry powder, averaging 3% by dry weight. The curry powder has smaller amounts of curcumin present, and the variability between different spices was great (Li et al., 2011).

Some studies have confirmed that turmeric or curcumin can increase the level of postprandial serum insulin, so eating some curries benefits diabetic patients

(Balasubramanian et al., 2012). Curcumin can also be produced by chemical methods, but the synthetic curcumin cannot be added to foods (Tawney and Penzer, 1924). Turmeric's antioxidant potential could be used in food industry and may substitute for synthetic antioxidants (Maheshwari et al., 2006).

Many western countries import turmeric from India every year (Table 3), but a huge part of this turmeric is not used as ingredient for food or for pharmacy. Another important utilization of turmeric, or curcumin is as a colorant. Curcumin is a healthy and stable food colorant that provides a bright yellow to orange-yellow with proper pH. The United States has industry to process turmeric into curcumin or oleoresin. But this amount is insignificant compared to the processed turmeric products that are imported from other countries directly (Brady, 1921). Curcumin appears in many foods including soups, baked food, drinks and ice cream. At the same time, adding curcumin as a colorant in food also keeps food fresh as preservative or antioxidant. An experiment showed that the shelf-life period of chicken breast increased by 39 days when curcumin colorant was added and processed with a proper dose of gamma radiation (Abdeldaiem, 2013).

Turmeric's Medicinal Value

Intact dried rhizomes, turmeric powder (ground turmeric), curcumin, oleoresin and volatile oil are the main ingredients used for medical purposes (Gounder and Lingamallu, 2012). Turmeric has a long history of medicinal use in South Asia and was widely used in Ayurvedic, Siddha and Chinese traditional medical systems (Bansal et al., 2008). There are more than 100 species of *Curcuma* and many of them used medically. The extracts from *Curcuma longa* are abundant; there are at least 20 compounds which are antibiotic, 14 are known as cancer preventives, 12 are anti-tumor, 12 are anti-inflammatory and there are at least 10 different anti-oxidants. In fact, 326 biological activities of turmeric

are known (Bansal et al., 2008). Turmeric has antiviral, antifungal, wound healing, and antimicrobial properties.

Many diseases like Alzheimer's diseases, diabetes, cirrhosis and colitis are caused by inflammation. Adding some components of turmeric into medicine can relieve the inflammation and treat these diseases eventually (Olojede et al., 2009). There are multiple pathways by which curcumin is effective against inflammation. First, curcumin decreases the production of inflammatory substance. Secondly, it can enhance or extend the reaction of the body toward inflammation, specifically, increase the secretion of adrenal hormone and cortisol. Third, curcumin can promote circulation of toxic substances which accelerate spitting out of them from inflamed tissues (Mishra and Palanivelu, 2008).

Curcumin shows anti-inflammatory, hypolipidemic and antioxidant properties and includes vitamins C, E and Beta-Carotene, giving it the potential to be considered in the development of cancer preventive strategies, liver protection and applications in clinical research. Based on animal and human clinical tests, it has been proved that curcumin is safe even at high doses, but curcumin has not got permit as therapeutic agent, maybe due to its poor bioavailability (Grewal et al., 2003).

Recent studies have shown that curcumin may contribute to the fight against cancers. Tumor cells use multiple pathways to protect them from the attack of host's immune system, but scientists have found curcumin can inhibit signal conversion pathway of tumor cells. In this case, tumor cells become fragile and could be easily

removed. Turmeric's other property, anti-angiogenesis, is also very useful (Ravindran et al., 2007). In the future curcumin may hopefully save cancer patients.

India started turmeric research in the 1950s on comprehensive subjects including botany, physiology and planting technology. This has helped scientists and farmers a lot. India has abundant cultivars adapted to different planting environments and that have high yield and good quality for multiple purposes. But for the new and promising area of pharmacology, many developed countries like U.S., U.K. and Japan have contributed a lot (Shetty et al., 1982).

Global Marketing of Turmeric

India is the biggest producer and exporter of turmeric (Table 1). Other major producer includes China, Myanmar, Nigeria, Bangladesh and Pakistan. Turmeric is an important commercial plant around the world but there are not many companies that provide specific data. The same item may have different statistics in different data source. Just like the second biggest turmeric output country varies from different studies. Data in Table 1 can just be regarded as illustrative only. India has the highest production and the longest cultivation history of turmeric, but Pakistan has almost the highest yield per unit area, even treble to fourfold that of India's (Ravindran et al., 2007). The higher production of turmeric in India, despite the higher productivity in Pakistan is explained by the greater planting area in India (Tahira et al., 2010). However, official and authentic figures are available in only a few countries. Some countries set turmeric as the standard

for reporting data, but other countries report turmeric as mixed processed spice which makes it difficult to obtain precise data on production, export and import of turmeric.

From Table 2 and Figure 1, it could be concluded that the export rate of turmeric in India is incredibly low. Different data sources are slightly different, but most of them are lower than 10%. That means that more than 90% of turmeric is used for domestic consumption. Interestingly, some western countries even export curcumin to India (Lal, 2012). Turmeric production in India is greatly expanding, but higher production has not contributed to an increase in the percent exported. This increasing production must reflect an increase in domestic demand, likely from a growing population.

Chemical Constituents of Turmeric

In general, the biggest component in turmeric is carbohydrates (69.4%), followed by moisture (13.1%), based on partially dried weight. There are also some proteins (6.3%), fats (5.1%), and minerals (3.5%). All data above are based on dry weight of turmeric (Bansal et al., 2008). To date, at least 235 compounds have been extracted from turmeric. These include 22 diarylheptanoids and diarylpentanoids, 8 phenylpropene and other phenolic compounds, 68 monoterpenes, 109 sesquiterpenes, 5 diterpenes, 3 triterpenoids, 4 sterols, 2 alkaloids, and 14 other compounds. Before curcumin was isolated, it was known in traditional medicinal systems that the rhizome of turmeric has valuable characters to cure ailment, but with the analytical theory development, people know that the main extracts of interest are curcumin and volatile oil (Tawney and Penzer, 1924; Lal,

2012). Now we know turmeric is a source of omega-3 fatty acid and alpha-linolenic acid (Masuda et al., 2001).

Curcumin makes up two to six percent of turmeric. Another three to seven percent is volatile oil (Bansal et al., 2008). Because of the volatile oil in rhizomes, turmeric has an aromatic flavor and smell (Garwal et al., 2003). Turmerone (11.1%) is the main volatile oil compound, and it has been demonstrated that it has the properties of neoplasm inhibitor and antivenom (Leong et al., 2008). In addition, eucalyptol (11.2%), turmerone (11.1%), caryophyllene (9.8%), ar-turmerone (7.3%) and sesquiphellandrene (7.1%) are all important components (Garwal et al., 2003; Maheshwari et al., 2006). Unlike curcumin, turmerone should be extracted from fresh rhizomes (Tayyem et al., 2006). On average, each turmeric plant can yield nearly 7.72 g volatile oil, rhizomes are the main source, but they are also present in leaves (Tayyem et al., 2006).

The volatile oil from fresh, dried and cured turmeric rhizomes was isolated and characterized chemically and its functionality with respect to its antioxidant potential was studied. The volatile oil extracted from dried as well as cured rhizomes showed a higher antioxidant potential than oil from fresh rhizomes (Tayyem et al., 2006). It has been reported that extracts from turmeric leaves, which include volatile oil, are useful to help drive away and kill mosquitos (Leong et al., 2008).

Cultivation

Turmeric needs high temperatures, between 20 and 30°C (68–86 °F), and sufficient water to grow normally (Mohammad et al., 2009). In turmeric's native planting area in Asia, it mostly grows in hot south and southeast Asia, and it has spread to several tropical and subtropical regions (Tawney and Penzer, 1924). In Okinawa prefecture, southern Japan, some field tests showed that turmeric rhizome seed did not sprout below 10°C or above 40°C (Ishimine et al., 2004). By increasing the temperature from 15°C to 25°C, sprouting was increased from 76 to 100% and the rate was constantly high between 25°C and 35°C (Figure 2). Above that point, the sprouting rate rapidly decreased to almost 0% at 40°C (Ishimine et al., 2004). Researchers have demonstrated that different environmental conditions may contribute to huge differences in curcumin content. Four varieties were tested in two locations in different provinces in India. At one location, one variety contained 60% of curcumin concentration than it did at another location. Other characters, like biomass and growth rate also differed by planting locations. Hence selecting proper variety for each planting area is an important consideration (Zachariah et al., 1999).

Each turmeric rhizome used for planting is about 30 g, giving a planting rate of about 2000 kg·ha⁻¹ (Kumar and Gill, 2010). Both mother and primary lateral rhizomes are used. The size of seed rhizome usually has important effect on the growth of the plant i.e. bigger seed always mean stronger plants. But this rule does not always work very well for

turmeric, i.e. bigger seed may not mean better. Thirty grams is sufficient weight to achieve rapid emergence (Kumar and Gill, 2010). But in some states of India, 4 cm is the suggested planting depth. Each planting depth standard should be based on specific soil type. Earlier emergence enables turmeric to grow faster than weeds, allowing it to grow taller than weeds. Taller turmeric suppresses surrounding weeds and that will significantly decrease the biomass of weeds compared to late sprouting, i.e. milder weed infection (Erulan et al., 2015).

In some countries, it is normal to plant turmeric with other crops; corn-turmeric-intercropping is the most common planting system (Avilkumar and Reddy, 2000). But these planting systems are likely to decrease the yield of each crop. There is planting pattern which consists of corn and turmeric, corn grows faster than turmeric, like a big cap and take much light from turmeric, which makes turmeric cannot get enough light to meet the requirement of photosynthesis. Besides, stronger corn can also absorb more nutrients and water from soil (Ishimine et al., 2015a). In all, corn-turmeric-intercropping system could bring high benefits in terms of total field production but may cause some loss in terms of turmeric production. The competition between weed and turmeric, corn and turmeric, but in the first one turmeric was the winner, in the second one turmeric was defeated (Nihayati et al., 2018). When just considering turmeric, we just need to find a suitable planting pattern only for turmeric i.e. monoculture of turmeric (Avilkumar and Reddy, 2000).

1 Planting Technology

Some papers give the standard planting preparation. In Japan, researchers and farmers plow the field deeply (30-40 cm), then prepare ridges spaced 1.5 m apart. Two rows spaced 30 cm apart are planted on each ridge with an in-row spacing of 20 cm (Ishimine et al., 2015). Turmeric may be grown on ridges to provide more abundant space for root to develop. Some experiments have shown that planting two rows on a bed about 100 cm wide gives the best results (Yamawaki et al., 2013). In addition, using a 30-cm-triangular planting pattern will help to get higher yield compared to most other patterns (Yamawaki et al., 2015). A 40-cm-triangular planting pattern gives lower yield and biomass of per plant due to lower density. When selecting 20 cm as the distance, the yield decreases significantly. Another strength of 30-cm triangular planting pattern is lower weed biomass compared to 40-cm pattern. Selecting 30 cm as the distance contributes to faster development of canopy-structure which inhibits the photosynthesis of weeds during the middle and later phases of growth. Longer distance between turmeric plants and a one-row-pattern has the opposite effect, intensifying the competition between turmeric and weeds (Hossain et al., 2015). An interesting thing is that some articles stated that intercrop pattern of corn and turmeric could protect turmeric during typhoons and results in minimum level of damage (Hossain et al., 2015).

After we plant the seed rhizome, the rhizome will sprout late and new shoots emerge after about 1 month. The weather during this stage cannot affect it too much. And then in

the second month rainfall makes a big difference. In the first month all the nutrients needed for sprouting come from the vegetative propagation tuber, then the percentage of nutrients from the seed rhizome decreases gradually. After 50 to 60 days, new plants can use the nutrients in the soil to keep growing. After this time, the shoots and leaves grow rapidly (Kandiannan et al., 2002).

2 Irrigation

Turmeric has a long period of growth and high demand of water. In general, higher level of irrigation always means higher Leaf Area Index (LAI), tillers and plant height (Wani et al., 1957).

Comparing drip and flooding irrigation, drip irrigation systems provide durable and light irrigation near the root. More water is absorbed directly by the root and quickly, contributing to less water loss. In addition, the low availability of soil water between turmeric plants makes it harder for weeds to grow, thus suppressing weeds like mulch. In terms of water productivity, drip irrigation system needs about 20 cm water less than the check basin method (Thiyagarajan et al., 2011).

The first overhead irrigation should be applied at planting. After that, irrigation could be scheduled. Turmeric has different water needs when grown in different environments. The irrigation water /cumulative pan evaporation (IW/CPE) system could be used to get a higher water productivity (Kaur and Brar, 2016). Suggested depth of drip irrigation is about 40 to 50 mm. From several experiments, irrigating at 1.2 IW/CPE

schedules can give good results in terms of higher growth and yield. The ratio of 1.2 IW/CPE means that for each month the amount of irrigation water should be 20 % higher than average evaporation water. But during the first two months the ratio of IW/CPE could be increased appropriately. Actual weather and other environmental conditions should be considered together with recommended irrigation standard (Kaur and Brar, 2016).

3 Fertilization

Fertility levels make a significant difference on plant growth. Adequate fertility helps plants develop lush vegetation and get high yield, i.e. expected biological yield and economic yield. Within the proper range, higher fertility level always means more powerful promoting effect.

Fertilizer can be divided into two types, chemical fertilizers such as urea, and organic manures such as poultry manure. Some experiments showed that proper rate and amount of chemical fertilizers can give pretty good yield (Sasikumar, 2005), but other studies showed that conjunctive use chemical fertilizers and organic fertilizers can get better results (Randhawa et al., 1973; Meerabai et al., 2000; Akamine et al., 2015). These two fertilizers have different characteristics and should be combined. Specifically, chemical fertilizers contain higher nutrient concentrations and are more available and readily absorbed. On the other hand, organic fertilizers can improve physical, chemical and biological properties of soil. Organic fertilizers provide plants a comprehensive

friendly environment that enhances the absorption of water and plant nutrients. One experiment showed that use of manure to provide 75 % of substitutable N resource and normal amount of P and K resource can get obvious increase.

Vermicompost, goat manure and poultry manure could be suitable sources of organic fertilizer. Inoculation of nitrogen fixing bacteria into vermicompost increased availability of N. These bacteria could increase turmeric yield, biomass and microbial population. Available bacteria source could be *Arthrobacter*, *Klebsiella*, *Serratia* and so on (Ponmurugan, 2012). In addition, vermicompost has a remarkable difference on the development of mycorrhizae (Kale et al., 1992; Kumar and Singh, 2001). Application of poultry manure or combination of poultry manure and NPK fertilizer significantly increased soil chemical composition and enhanced growth parameter and vigor during two seasons (Adeniyi and Ojeniyi, 2005; Dauda et al., 2008).

During different phases of the growth cycle, turmeric has different nutrient demands. During the rapid growth phase, turmeric needs to uptake more nutrients. Specifically, turmeric needs more potassium during the first three months, nitrogen during the first four months, and phosphorus during the first five months. After that, the demand will decrease gradually (Ravindran et al., 2007). Common deficiencies often occur for macronutrients like nitrogen, phosphorus and potassium.

The use of organic fertilizers may cost more than use of chemical fertilizers due to more labor needed, but more economic benefit which higher yield brings can easily cover

the cost (Jayaprakasha et al., 2001).

Although micronutrients are not so abundant as macronutrients, some of them also are essential for plants. For example, iron involves various physiological processes like photosynthesis and electron transportation. The deficiency of iron, zinc and boron are the most common problem (Babu, 1989).

The typical symptom of iron deficiency is chlorosis, which always appear in young leaves first. The leaves could be entirely white due to the absence of chlorophyll, which could be destructive. Iron is key because it is the central element to synthesis of chlorophyll. Iron deficiency is a common deficiency in some calcareous and alkaline soil (Vastava and N., 2000). Too much phosphate in acid soil could also induce iron deficiency. To prevent this deficiency and help turmeric grow, farmers could apply FeSO_4 ($30 \text{ kg}\cdot\text{ha}^{-1}$) as a foliar spray of 0.5 % FeSO_4 in the third, fourth and fifth month after planting (Dixit and Vastava, 2000).

Zinc deficiency is also very common in turmeric planting and it was regarded as the most widespread micronutrient disorder of food crops in India and the world, particularly in arid and semiarid regions where alkaline soils predominate. The data showed that 47% of Indian soils were found to be deficient in zinc. The main symptoms are light green, yellow and white tissues between the veins of leaves in older leaves. In addition, it could result in narrow leaves, early dropping of leaves and stunted growth. To prevent this deficiency and help turmeric grow, farmers could apply ZnSO_4 (15 kg/ha) (Clarkson and

Hanson, 1980).

When boron deficiency occurs, farmers could use borax to treat it. Using a micronutrient mix fertilizer could be an easy choice. Ravindran et al. (2001) recommended a micronutrient mix made up of 375 g FeSO₄, 375 g ZnSO₄, 375 g borax, 375 g urea and 15 kg super phosphate in 250 L water·ha⁻¹ applied as a foliar spray with a second application 25 days after the first treatment. Although some experiments proved that light iron and boron deficiency could increase the curcumin content, this may lead to a drastic decline in yield, such that these deficiencies are not desirable (Ravindran et al., 2007).

In western Maharashtra, India, application 200 kg N ha⁻¹, 100 kg P₂O₅ ha⁻¹ and 100 kg K₂O ha⁻¹ gave the best biological performances of turmeric and the highest yield on medium-deep black soil. Besides, split application of fertilizer at 6, 12- and 18-weeks days after planting made improvement (Yamgar et al., 2001).

In a field experiment in Coimbatore district, India, application of 150 kg N ha⁻¹, 60 kg P₂O₅ ha⁻¹ and 108 kg K₂O ha⁻¹ was used as recommended fertilizer application on a sandy clay loam soil with pH 8.2 (Jagadeeswaran et al., 2005). Also, the authors recommended the use of tablet forms of NPK nutrients rather than traditional granular types (Jagadeeswaran et al., 2005).

In Allahabad, India, a nutritional trial concluded that application of 75 kg N ha⁻¹, 60 kg P₂O₅ ha⁻¹, and 150 kg K₂O ha⁻¹ is the best combination on clay loam, low in nitrogen

and potassium, medium in phosphorus and a pH of 7.4 soil (Thomas et al., 2002). In Shillong, India, on red lateritic soil, the highest yield and curcumin content was obtained with the combination of straw mulching plus 120 kg N ha⁻¹, 60 P₂O₅ ha⁻¹, and 160 kg K₂O ha⁻¹.

In Karnataka, India, on medium deep black clayey soil with soil pH of 8.3, a field experiment was conducted to assess the best fertilizer rate in 2012 with drip irrigation system. Application of 270 kg N ha⁻¹, 135 P₂O₅ ha⁻¹, and 180 kg K₂O ha⁻¹ contributed to the best growth and yield compared to other fertilizer treatments (Satyareddi and Angadi, 2014).

As mentioned above, common inorganic chemical fertilizer is not the only choice. Singh (2011) used farmyard manure (FYM) and *Azospirillum* as organic and bio-fertilizer, at a rate of 5 Mg FYM ha⁻¹. He concluded that combination of organic, inorganic and bio-fertilizer also contributed to high yield (Singh, 2011).

4 Plant Growth Bio-regulators

Bio-regulators play an important role for growth of plants and complete life cycle successfully like inorganic nutrients. For example, some articles stated that auxin plays an important role in protein synthesis, can reinforce photosynthesis and other essential plant physiological processes (Woodward and Rtel, 2005). Some articles proved that using 1-Naphthaleneacetic acid (NAA) as plant growth regulator gave obvious beneficial effects on the growth and development of turmeric (Nasirujjaman et al., 2005).

Gibberellic acid (GA₃) was shown to give similar effects. Unlike NAA and GA₃, Paclobutrazol will decrease plant height but still could contribute to rhizome yield (Satheesan et al., 1988). Experiments showed that applying Paclobutrazol (0.2 and 0.4%) led to 53 and 60% reduction of growth, but for underground parts, the number of three kinds of finger rhizomes increased a lot. Even though the length of rhizomes decreased, the final yield was still 23 and 38% higher than the control group. In addition, Paclobutrazol can enhance the development of xylem and phloem, which will help increase the number and size of oil cells and curcumin cells (Wang et al., 1986). That means that farmers could get high yield with wonderful quality with Paclobutrazol application (Satheesan et al., 1988). These plant growth bio-regulants may be applied as foliar spray at nearly 90 days after planting (Olojede et al., 2009).

5 Mulching and Shading

Many experiments have shown that mulching significantly improved the yield of fresh and cured rhizomes compared to no mulching. In all, mulching has a higher benefit cost ratio (B/C) than without mulching. Some experiments demonstrate that mulching can increase the rhizome yield by 59.2 % and 218 % when using straw mulch (Manhas et al., 2011; Satyareddi and Angadi, 2014). These favorable influences mostly result from higher water retention and availability in the soil and earlier emergence. Mulching has been shown to hasten turmeric emergence, which means mulching will leave more time for turmeric to grow and mature. As a result, farmers get better and more turmeric.

Application of mulch contributes to reduced soil evaporation, especially in dry period. Mulching with straw, paddy husk, or plastic film can suppress weeds, decrease the appearance of weeds and delay the germination of weeds by limiting photosynthesis of weeds, improve microflora and fauna in soil, and protect soil surface from raindrop splash (Manhas et al., 2011; Satyareddi and Angadi, 2014). Generally mulching should be placed just after application of the herbicide (Kaur and Brar, 2016).

Using shade may help turmeric grow luxuriantly, but higher yield and better rhizome may be obtained when planted in open ground (Ridley, 1912). Other field experiments showed that shade gave the highest yield and some pivotal substances like curcumin and oleoresin (Padmapriya and Chezhiyan, 2009; Padmapriya et al., 2016). One interesting test conducted in India by Sarangi et al. (2007), showed that high turmeric yield and the concentration of curcumin, oil, oleoresin and chlorophyll were obtained under the shade of different trees. However, they did not compare this with no cover plant group (Sarangi et al., 2007).

6 Weed Control

The growth of turmeric needs a high level of irrigation, which can also favor growth of weeds. Weeds will compete with turmeric and other crops for nutrients, moisture, light and space (Kaur et al., 2008). Hence, weed infestation is one of the most important factors that will influence the development and yield of turmeric. The first weeding should be done just after planting turmeric. A second weeding may be done at 30 days

after planting, when about 60 to 70 days after planting, weeds cannot compete with turmeric (Sachdeva et al., 2015).

Some articles stated that the loss of yield resulting from weed infestation (45%) is higher compared to the pest (30%) and diseases (20%) (Kaur et al., 2008; Manhas et al., 2011). When there is drought, this situation could be worse, because when weeds compete water with turmeric, naturally they are always the winners. Weeds are better able to compete for water than turmeric. In this case, too much or insufficient water are both likely to favor weeds and threaten most crops in the field. What's more, some types of weeds stay in the field all year, providing a safe and durable host for harmful pests and diseases. Weeds help these enemies survive tough period. Weeds can help the pests, their eggs and pathogens survive during cold winter, so that people need to fight with them year after year (Tahira et al., 2010).

The dominant weed flora will vary with soil type and climatic conditions. Also, different seasons always have different dominant weed type in same field. There are several methods to control weeds, which rely on labor or technology. Traditional weeding methods are not efficient compared to modern ways, but they still cannot be replaced totally. Specifically, different methods have different costs and benefits, human should choose suitable methods or combined methods to settle their weed problems in their fields. For some developing countries, weeding is still carried out manually, but in other countries with better financial condition, like China, use of chemical herbicides has

occupied main markets due to low cost. However, in some western countries, organic food, i.e. the health of food, is more important than the yield. In these rich countries, integrated weed management or total organic approach are more acceptable for farmers and customers. Choosing which kind of method depends on practical situation.

Purple nutsedge (*Cyperus rotundus*), a troublesome weed over the world, especially in tropical and subtropical belts, affects more than 90 countries and 52 crops (Bendixen and Nandihalli, 1987). Purple nutsedge has been serious in some areas with high temperature and moisture. Purple nutsedge rarely reproduced by seed. Like turmeric and other underground plants, it could use tubers to reproduce (Wills and Briscoe, 1970; Wills, 1987). Once purple nutsedge appears in field, the density will normally be very high. In this case, weeding organically is labor-intensive. Besides, weeding deeply likely brings damage to turmeric roots, broken roots of purple nutsedge even help them distribute faster (Hossain et al., 2008).

There are some other common weeds in traditional turmeric fields, like sensitive plant (*Mimosa pudica*), slender amaranth (*Amaranthus viridis L.*), southern crabgrass (*Digitaria ciliaris Koeler*), spiny amaranth (*Amaranthus spinosus*), spiny sowthistle (*Sonchus asper*) et al. (Hossain, 2005; Hossain et al., 2008). Some herbicides may be used to deal with these weeds in turmeric field: alachlor, dichromate, nitrofen at 1.5 to 2.0 kg·ha⁻¹ as preemergence herbicides. In addition, metribuzin at 0.70 kg·ha⁻¹, diuron at 1.0 kg·ha⁻¹ (Ravindran et al., 2007) or butachlor at 1.5 kg·ha⁻¹ are all effective on controlling

weeds (Babu, 2008). Sometimes we need to combine herbicides to achieve the best effect (Sachdeva et al., 2015).

As we all know, the application of herbicides always contributes to phytotoxicity. It is the main side-effect of these chemical substances. Besides, considering about the medical value of turmeric, normally people use organic methods to grow turmeric.

7 Insect and Pests

There are about 60 to 70 species of insect pests that infest turmeric fields. In India, shoot borer (*Conogethes punctiferalis* Guen.) and rhizome scale (*Aspidislla hartii* Sign.) are the most common (Velayudhan and Liji, 2003). Leaf pests have been reported, such as skipper, lacewing bug and leaf beetles. These pests are more common in some drier regions (Arutselvia et al., 2012). *Stephanitis typicus*, *Panchaetothrips indicus*, *Orthacris simulans*, *Letana inflata*, *Conogethes punctiferalis*, *Udaspes folus* and *Mimegralla coeruleifrons* were reported as important pests in turmeric field (Kotikal and Kulkarni, 2000), as well as cigarette beetle (*Lasioderma serricornis* Fab.), drugstore beetle (*Stegobium paniceum* L.), and coffee bean weevil (*Araecerus fasciculatus* DeG) (Ravindran et al., 2007).

Among these pests, shoot borers are distributed over Asia, Africa, America and Australia. In organic turmeric production, they can be controlled using natural enemies including viruses and parasitoids, sex pheromones and biopesticides (Kotikal and Kulkarni, 2000a).

Rhizome fly also may appear in turmeric and ginger fields but just as a secondary pest. There are three main rhizome fly types, *Calobata sp.*, *Calobata albimana Macq.* and *Mimegralla coeruleifrons Macq.*, but up to now, only *Mimegralla coeruleifrons Macquart* has been reported to infest turmeric field (Kotikal and Kulkarni, 1999). The total life span from 38 to 62 days, the peak of infestation is from mid-August to mid-October. The infective period is the pupal stage. Insecticides are useful to control this pest (Ghorpade et al., 1988). All this information is from India; none were available source from other countries.

Some pests also attack stored dry turmeric rhizomes, which decreases final yield (Arutselvia et al., 2012). To prevent storage losses, fumigation, heat treatment, radiation or insecticides may be used. Rhizomes should be checked carefully (Pruthi, 1992).

8 Diseases of Turmeric

There are several diseases that can affect turmeric. Rhizome rot and foliar diseases are in dominant positions and could lead to severe loss. The type and degree of diseases vary much due to different planting regions and local growing conditions. Different diseases have different pathogen but most are caused by fungi.

a. Rhizome Rot

According to Anusuya and Sathiyabama (2015), most rhizome rot disease is caused by *Pythium aphanidermatum* and other *Pythium* species. Some species of *Fusarium* are involved in rhizome rot. *Pythium aphanidermatum* (Edson), *Pythium graminicolum*

(Middleton), *Pythium*, *Fusarium solani* and *Pythium myriotylum* (Assam) are the main pathogens. These fungi are distributed around the world and can be found in many soils with pH 3 to 9, but the best environment for them is between pH 7 to 8. One article reported that maggots of *Mimegrella coerulifrons* are the primary agents of this disease, and when the rhizome is cut, these maggots are found inside (Rao, 1995). Meredith and other researchers in Auburn University first reported the Binucleate Rhizoctonia AG-G on turmeric in the United States (Meredith et al., 2018).

One of the symptoms of rhizome rot in aboveground parts are progressive yellowing of leaves. This first occurs along the leaf's margins, then the color occupies whole leaves, and finally yellow parts gradually become dry (Anusuya and Sathiyabama 2015a). Aerial shoots may also have water-soaked soft symptom. For underground organs, the disease first causes a little decay in the roots, which change to brown. Then the pathogens can readily infect the rhizomes. When rhizomes are infected, both mother and finger rhizomes start to rot and become soft with different shades of brown. Severe rhizome rot will kill the entire plant and emit a foul smell that can be noticed before harvest. The pathogens can spread in soil, so flow irrigation can help accelerate their propagation. New research showed that foliar application of β -D-glucan nanoparticles (GNPs) can help to control the rhizome rot (Anusuya and Sathiyabama 2015b).

Specifically, healthy rhizomes from disease-free locations should be selected as seed rhizomes. Another straight and effective pathway is using tissue culture.

Crop rotation and intercropping could give better growth and yield performance.

Common intercropping examples include turmeric-maize and turmeric-chilies. However, sometimes intercropping may decrease the yield of each crop in same field and result in lower yield and profit from turmeric, but this does not mean low overall benefits to the farmer (Sachdeva, Kumar and Rana 2015; Avilkumar and Reddy 2000; Khan et al. 2015; Behera et al. 2008).

Many dominant turmeric cultivars are susceptible to rhizome rot, but there are some varieties that show different level of resistance to *Pythium* rhizome rot, including PCT-13 (Suguna), PCT-14 (Sudarsana), Cv. Shillong, Ca-69, Ca-17/1 and Ca-146/4. These varieties could be regarded as germplasm resource to create new better varieties (Ramakrishnan and Sowmini, 1955; Rathaiah, 1982a; b).

Chemical substances always are the cheapest and most powerful tool to control most diseases, weeds and pests in field, but on the other side they are likely to produce unhealthy or unsafe foods and leave pollution behind.

b. Leaf Blotch

Leaf blotch, *Taphrina maculan*, is caused by a common fungus in the main turmeric-growing regions (Upadhyay and Pavgi, 1967). The first infected leaves appear as pale-yellow spots on upper surfaces, less often on lower surfaces. The color will change to dirty yellow without control and finally deepen to the color of old gold and sometimes to bay shade. Each spot is small, about 1 to 2 mm in diameter and discrete (Rao, 1995; Rao

et al., 2012). Hundreds of spots consist of a huge blotch, and the infected areas are distorted and occupied by reddish brown blotches when the infection occurs seriously. Mostly these show on lower leaves (Upadhyay and Pavgi, 1967).

The appearance and severity of leaf blotch mostly depend on the concentration of pathogens in the soil. There are two stages in which turmeric is susceptible to this pathogen. The first one is when temperature is at 21 to 23 °C, another is later under cool and humid conditions (Upadhyay and Pavgi, 1966).

c. Leaf Spot

Leaf spot is another prevalent foliar disease for turmeric and was recorded mostly in turmeric-growing areas, but the intensity of disease varies from each other. The main pathogen of leaf spot disease is *Colletotrichum capsici*, which can cause severe loss in yield (Jagtap et al., 2013). The leaf spot disease may happen when the humidity and temperature is too high, and that provides this fungus with fitting environmental conditions to develop. The symptoms could be noticed mostly in new leaves. In general, the pathogen attacks the upper surface, but it can also be observed in both surfaces under severe situation. The infection is mostly confined to leaf blades, but occasionally shows on leaf sheaths and turmeric flowers (Narasimhudu and Balasubramanian, 2002).

Initially, each spot is elliptic or oblong with variable size, and is separate from others, and measures up to 40 mm in diameter. After that, spots increase their size quickly and coalesces with other spots, show irregular shapes that eventually dry up. Each

combined spot has grayish white and thin center with many black dots on both surfaces. There is a brown margin surrounding the center (Mina et al., 2009). A lot of hosts provide desirable space for the pathogens to live. Infected leaves are the main source or host. Some experiments have shown that the pathogens can remain viable in a buried leaf for 3 months and survive up to 1 year. Other plant species including pigeon pea, sorghum, ginger and pawpaw can also serve as hosts for this pathogen. Hence, removing turmeric and other crops leaves from field is an essential means to control leaf spot disease in these plants. Having pathogen-free seed rhizomes and soil environment also is the key to prevent leaf spot as well as leaf blotch. Some articles indicate that proper shade also has a positive influence. However, as reported above, intercropping may decrease the yield and profits. People need to consider loss and benefits of shade in practical production (Gorawar et al., 2006).

Recommended chemical agents to control leaf spot are Bordeaux, sprayed at 1 % Bordeaux once in early August, as well as Captan, Dithane Z-78 and others (Ravindran et al., 2007).

9 Harvest

As an annual crop, turmeric usually could be harvested at about 8 to 9 months after planting, depending upon the variety, climatic and soil conditions. Before the first frost comes the turmeric stops its main physiological activities and stores or withdraws most nutrients into rhizomes. The maturity could be judged by the leaves turning yellow, then

showing white and becoming totally dry. Generally, leaves and shoots turn to yellow when daily average temperature is below 20 °C. Some experiments have shown that the fresh weights of turmeric were similar in November, December and January. However, the dry weights of rhizome showed huge differences depending upon harvest date. Later harvest likely could contribute to higher rhizome yield. This means that we need to give turmeric enough time to transport the most nutrients to the rhizomes to obtain maximum yield. According to Hossain (2015), turmeric should be harvested in spring but the specific date should depend on local conditions (Hossain, 2015).

Harvesting turmeric manually always means drudgery, which requires 120-130 woman days per ha. This also means low productivity. Also, farmers or workers could miss some rhizomes, resulting in loss. A powered harvester would be more efficient (Thankamani et al., 2013). It was recorded that a primary harvester was designed at about 2002, but based on the main articles published, part of the harvest usually was done by hands (Ravindran et al., 2007). Now, existing turmeric harvesters consists of four parts, gear box, digging blade, conveyor and collection box (Annamalai and Udayakumar, 2007). A problem with these harvesters is that they result in more yield loss compared to manual harvest, and the harvester should be adjusted for different fields. Much improvement is needed before mechanical harvesters are widely accepted.

When harvesting, the first step is to cut the leaves and stems close to the ground i.e. keep as little aboveground parts as possible. Then dig the rhizomes. There likely is not

real mature harvester for turmeric up to now, which makes the harvest highly dependent upon labor. After that the rhizome from each plant should be separated, and mother rhizomes separated from finger rhizomes. Finally wash each one carefully.

10 Storage

The preservation of rhizomes is one of the most essential things for planting turmeric because most farmers will use about 15 to 20 percentage of rhizomes harvested as “seed”. The quality of rhizome is the first important challenge for all crops that farmers need to face because the yield of crop always is the most important target.

It is important to understand the reasons why storage is so important for seed rhizomes. Jayakumar et al. (2001) found that the content of some essential substances including protein, RNA and nucleic acids increased gradually starting at 15 to 30 days after stored. And this process enhanced rapidly around 75 days during storage. These substances play a dominant role in sprouting in other rhizome and tuber plants (Jayakumar et al., 2001). This finding may also apply to turmeric but has not been proven (Jayakumar et al., 2001).

The following methods of turmeric rhizome storage has been reported in the literature:

- 1.) Stored in pots lined with saw dust in shade in specially made cubicles to avoid heat and light (John and K, 1992);
- 2.) A pit of 60 cm deep and large enough to hold the seed rhizomes is dug in a cool,

shady, dry location. Covered lightly with loose, dry soil and turmeric, banana, or other leaves (Ravindran et al., 2007);

3.) Seed rhizomes stored under shade and covered with turmeric leaves plastered together with mud and cow dung (Ravindran et al., 2007);

4.) Turmeric seed rhizomes could be stored in zero energy chamber with minimum storage losses due to physiological loss in weight, rotting, and insect damage (Ravindran et al., 2007);

5.) Rhizomes for consumption and processing may be dried and be stored under ambient room conditions in polyethylene bags with 0.5 % ventilation in a dry and cool room; If under field conditions, rhizomes may be stored in pits lined with wheat straw (Ravindran et al., 2007).

Some experiments have demonstrated that the content of the most important constituents, curcumin and volatile oil, didn't change or only changed slightly regardless of how long or short duration of storage under suitable storage processes (John and K, 1992; Ravindran et al., 2007).

11 Postharvest Technology and Processing of Turmeric

After good cultivation and pre-harvest treatments, these post-harvest methods also play an important role on final yield and quality. Freshly harvested turmeric rhizomes have a lot of moisture, soil, stem and other parts and contaminants that must be removed, so processing is essential in order to keep harvested rhizomes as seed or process for

commercial products.

The conventional processing consists of curing, drying and polishing to be sold as dried turmeric rhizomes, while another pathway consists of slicing, drying and grinding to be sold as turmeric powder. These mostly target different commercial purposes. Besides, in different papers researchers held different definitions for these processes. Like some “washing” also includes boiling, or combined several sections into one part, like polishing. Here we just give the basic and common definition of main processes. This review only includes the first system.

a. Curing

The main part of curing is cooking cleaned rhizomes in boiled water until they are cooked, so the curing also could be called “cooking”. Prior to cooking, the rhizome bulbs and fingers should be separated as well as other foreign mass like soil. The rhizomes can be washed if necessary. It is not advisable to remove of the peel because that will bring enormous loss of final mass. Next is cooking. Cook rhizomes in boiling water for 30 to 60 minutes depending on the quantity and other factors of turmeric. Soft rhizomes and froth mean cooking is finished. There are some conflicts here, two choices, one is to use plain water and another one is alkaline water with added sodium bicarbonate or carbonate. It was said that alkaline water helps improve the color (Ravindran et al, 2007). Color is one of the most important characteristics of turmeric products. However, other studies showed that, the content of pigments in final product was decreased by 9%

when cooked in alkaline water (Bambirra et al., 2002). The decrease should result from the sensibility of pigments to pH (Tonnesen & Karlsen, 1985; Souza et al., 1997). The processing will significantly affect the level of pigments in final product. Some experiments showed that cooking in plain water should be a better choice for producing ground turmeric (Ravindran et al., 2007).

Cooking it better to be finished with 2 to 3 days after harvest. Cooking prior to drying has several positive effects, promoting gelatinization of the starch, facilitating and increasing the dehydration rate of turmeric rhizomes and uniform distribution of pigments (Bambirra et al., 2002).

b. Drying

Drying is the core of whole process to make turmeric products. For example, The United States Food and Drug Administration (USFDA) has a specific standard of moisture content for whole turmeric. The maximum allowable moisture content is 10% in order to ensure shelf life of the product. (Jose and Joy, 2009). And the price of turmeric products, to a large extent depends on the moisture content.

There are several available choices to dry turmeric rhizomes, conventional sun drying, solar tunnel drying and mechanical drier. Different methods have different results i.e. different final moisture content. Producers should choose suitable methods based on actual situation including both the requirement of buyer and the condition of producer. For India, the biggest producer and customer, open sun drying is the traditional and

conventional method (Delfiya et al., 2014).

For sun drying, the cooked rhizomes should be spread on a proper drying floor like bamboo mats or Kraft bags (Prasad et al., 2006). Now it is time to select drying method. There is no doubt that open sun drying the most convenient but also the worst method; the effect mainly depends on local weather and it may require the longest drying time compared to other methods. However, when concerned about local financial condition this method is still popular in India. Solar driers make up for this shortcoming to some degree and increase the temperature inside, but it also cannot be a standard during commercial processing. Using mechanical drier can make sure the final products could reach the international standard moisture content of 10% mentioned above. Mechanized drying can reduce the drying time at a maximum temperature of 60 °C (Spices Board, 1995). Conventional drying needs 10 to 15 days (Anon, 2002). When moisture content at 5 to 10 % is reached, drying has finished. Extended drying may lead to the loss of valuable substances like volatile oils. Some pigments in turmeric also are sensitive to light, so mechanical drier also help to save them. Before the rhizomes have been dried totally, do not put rhizomes in polyethylene bags that may make it easier to happen mold. Slicing the rhizomes before drying may be an improvement to reduce drying time and extract valuable substances but that likely affect the content of volatile oil and pigment (Bambirra et al., 2002).

c. Polishing

Polishing mostly is the duty of dealers, not the growers, and polishing is only required for whole turmeric rhizomes products. Polishing would be done after drying the turmeric rhizomes to remove unwanted impurities. This can be done manually or mechanically. Polished turmeric rhizomes are smooth and shining, which give a better price (Yadav et al., 2013). To complete this purpose, the outer dirty skin, roots and soil particles should be removed during polishing. The traditional hand polishing method may consist of placing the finger and mother rhizomes in a gunny bag and rubbing or trampling them. The slightly developed methods use barrel or drum made of expanded metal mesh, operated by hand. Polishing machines vary according to different papers, some of them could be assembled easily and accessories are easily available normal market (Nair, 2019).

The phenomenon of abrasion used in polishing, which is caused by the friction between expanded wired metal mesh and turmeric. Some polishing machines can polish 50 kg dried turmeric rhizomes within only 20 minutes. Some papers regarded polishing as one deep washing, for these fresh harvested rhizomes, this kind of washing is not limiting to removing but also achieve some polishing purpose (Moghe et al., 2012).

12 Factors Influencing Turmeric Growth and Development

Some experiments demonstrated that turmeric resulting from mother rhizome are more robust both in emergence and development stages and result in higher yield and

curcumin content (Aiyadurai, 1966). Just like plump seeds result in more vigorous seedlings, mother rhizomes may give rise to more vigorous plants than finger rhizomes due to more abundant nutrient storage (Olojede et al., 2009). Normally turmeric needs 8 to 20 days to germinate after sowing. Experiments proved this standard works for most of species. After 20 days, the remaining seed rhizomes which still have not germinated likely have no vigor to germinate even later (Nambiar et al., 1982).

Curcuma is a big genus. Some special species not only have flowers but also have real seed. These species need proper environmental conditions to develop pollens and fertilize. Some projects have explored the best conditions for flowering and fertilization of these special species, such as temperature and pH. And for these special species, farmers could use real seed set to sow for next planting rather than seed rhizomes. Compared to rhizomes, seed requires shorter time to germinate but the growth rate is slower than rhizome (Udomdee et al., 2007). The first synthetic seed of turmeric was reported in India in 1997. The somatic embryos and shoot buds were encapsulated in calcium alginate. The artificial seed could keep vigor for seven months (Sajina et al., 1997).

The planting time of turmeric should be adjusted to coincide with local weather conditions. Mostly it relates to rainfall because turmeric demands much water during vegetative growth stage. Another important factor is the local temperature, as turmeric is a tropical plant turmeric that needs abundant heat and daylight. It is hard to get a standard

of planting time due to different varieties, materials and climatic conditions. Some experiments in India showed that planting during the first fortnight of June can give high yield and high curcumin content (Sasikumar, 2005).

13 Crop Improvement and Tissue Culture

Unlike other common crops, it is difficult to use hybridization to improve turmeric cultivars. Because most of turmeric varieties cannot develop real seed, clonal selection using tissue culture almost is the only way to improve the cultivar quality. Scientists used some explants like vegetative buds, rhizome bits or callus derived from buds & leaf, cultured in vitro with diverse culture mediums. Different combinations of explant and culture medium could get different clone products. Like callus derived from buds could regenerate shoot, but callus derived from leaf could regenerate new plant, but other tissue sources do not give the same results (Shetty et al., 1982; Praveen, 2005). And these are both based on different culture mediums. The main selection targets are high rhizome yield, high curcumin, essential oil and oleoresin contents, and resistance to some disease, like rhizome rot and leaf blotch. Based on the record, high fresh rhizome yield could be around $35 \text{ t}\cdot\text{ha}^{-1}$, some varieties could reach over $50 \text{ t}\cdot\text{ha}^{-1}$ in selected area (Jalgaonkar et al., 1988). Dry rhizome yield is normally lower than $10 \text{ t}\cdot\text{ha}^{-1}$. Seven or eight ton per hectare could be regarded as good performance (Pujari et al., 1986).

Some breeders used mutation breeding methods to obtain some good varieties. Available pathways are ionizing radiations like X-rays and chemical mutagens, like

ethylmethylsulfone (EMS) (Anonymous, 1986). Gayatri et al. (2005) created some root rot resistant varieties with somaclonal variation technology (Ishimine et al., 2015b).

Micropropagation by in vitro microrhizomes is the best solution, especially for turmeric, which cannot produce seed. MS is the most common medium, and supplied with N-(Phenylmethyl)-9H-purin-6-amine (BA), 1-naphthylacetic acid (NAA) and ancymidol (Rajan and V., 1997). And this method also could be used for hybridization, finish pollination and hybridization in vitro, regenerate plantlet and get new hybrid varieties (Renjith et al., 2001).

Justification and Objectives

Turmeric has potential as a high value crop for Alabama. It is a new crop and information is lacking on the best varieties to grow. The objectives of this research are to select turmeric varieties adapted to Alabama with high curcumin yield, high curcumin concentration and high rhizome yield.

Chapter 2 Turmeric Rhizome Yield

Introduction

The genus *Curcuma* is composed of approximately 110 species, many of which have been shown to have various uses. *Curcuma longa* (L.) is the most important and famous species, generally known as turmeric. It is a rhizomatous herbaceous perennial plant and belongs to the ginger family, *Zingiberaceae*. Currently, more than 80% of turmeric is produced by India and turmeric products are sent to numerous countries. Turmeric has primary and secondary rhizomes and roots. The fleshy, branched, inter laced rhizome makes turmeric difficult to be harvested by reapers (Maheshwari et al., 2006). Its rhizomes always have an aromatic property with yellow to brown color and 5 to 8 cm long, 1.5 cm thick, generally color of inside part is deeper. The primary rhizome is called as “mother rhizome” or bulb, pear-shaped in the center. The branch of mother rhizome is the secondary rhizome, called “finger rhizome”. Just like ginger, turmeric finger rhizome only germinates on a main axis. The shape of the rhizome is conical, hemispherical and cylindrical (Jayaprakasha et al., 2001). Available seed rhizomes are single mother rhizomes or complete finger rhizomes. But finger rhizomes are more commonly used in commercial production than are mother rhizomes (Jayaprakasha et al., 2001). The top of mother rhizome develops the above-ground shoots and leaves. Finger rhizomes sometimes enlarge and branch and then become new mother rhizomes. The first-order branches are known as primary fingers; new branches develop from them and are called

secondary fingers; and then there are tertiary fingers. Some of nodes on primary finger are the future secondary fingers. Besides the mother rhizome, some part of first fingers also could develop shoot. Others show positive geotropic growth with all secondary and tertiary fingers (Maheshwari et al., 2006).

For food, industry and medicinal utilization, fresh and dry rhizome yields are among the most important characteristics for successful turmeric varieties. But turmeric is a new crop for America, farmers and researchers lack information on the best suitable varieties. The assessment of turmeric yield mainly consists of two parts: fresh rhizome yield and rhizome dry weight yield. Fresh turmeric rhizome normally sold as vegetable and spice in grocery and farmers markets. Fresh rhizome also could be dried, which could be stored longer, and sold as a spice. Drying is an important step for further process and utilization.

Intact dried rhizomes, turmeric powder (ground turmeric), curcumin, oleoresin and volatile oil are the main ingredients used for medical purposes (Gounder and Lingamallu, 2012). Many diseases like Alzheimer's diseases, diabetes, cirrhosis and colitis are caused by inflammation. Adding some components of turmeric into medicine can relieve the inflammation and treat these diseases eventually (Olojede et al., 2009).

Turmeric has potential as a high value crop for Alabama. It is a new crop and information is lacking on the best varieties to grow. Based on different purposes of utilization, our research objectives are selecting good performance of turmeric varieties with high fresh rhizome yield, high rhizome dry weight yield, high curcumin

concentration and high curcumin yield. High curcumin concentration and yield are regarded as additional selection criteria.

Experimental Methods and Materials

Because turmeric varieties come from various sources and we do not know their names, the varieties were given accession numbers. The origin of the varieties is listed in Table 5.

Field trials were conducted at Auburn Agronomy Farm (32° 59' 35.4816" N, 85° 49' 50.8152" W) during 2016, 2017 and 2018. Soil was tested prior to fertilization in each season. Experimental soil is acid soil, sandy clay loam soil type with 1 to 6 % slope. Soil tests result given in Table 6, pH values ranged from 5.7 to 6.1.

There was a three-year process of elimination to arrive at the varieties that best met the objectives of the research. In 2016, there were eight turmeric varieties involved. Data collected on rhizome yield and curcumin content in the 2016 trials was used to select high performers to include in the 2017 and 2018 trials based primarily on curcumin yield. In 2017, variety CL10 was added to the six varieties retained from the 2016 trial. Harvested, collected data and selected in the same way. Four varieties were saved and planted in 2018.

Plant Establishment

Turmeric rhizomes were washed, surface air dried and treated in hot water at 45 °C for 50 minutes to kill any nematodes or nematode eggs on the rhizomes. Healthy, plump,

and uniform turmeric rhizomes were planted in 10 cm pots at the Auburn University Plant Science Research Center with potting mix (Sun Gro Horticulture Canada Ltd., Vancouver, British Columbia) on propagation mats on April 4th, 2016; April 7th, 2017; April 11th, 2018. The temperature of the mats was set at 27 °C. A drench of RootShield Plus+ WP (BioWorks), a biological fungicide, was applied to potted turmeric rhizomes before sowing in greenhouse to protect turmeric roots from some pathogens, like *Pythium*, *Phytophthora*, *Rhizoctonia*, *Fusarium*, *Cylindrocladium* and *Thielaviopsis*, and *Sclerotinia homeocarpa*. Active ingredients include *Trichoderma harzianum* strain T-22 and *Trichoderma virens* strain G-41. Only fertilizers and fungicides approved by Organic Materials Review Institute (OMRI) were used according to soil test needs. RootShield was applied by Hozon siphon mixer, 9.5 g l⁻¹. Pots were filled to the rim with solution. Potted rhizomes were saturated twice.

Field Preparation

As for fertilization, experimental fields received 1.5 tons of poultry litter prior to transplanting, which provided approximately 101 kg N ha⁻¹, 101 kg P₂O₅ ha⁻¹ and 67 kg of K₂O ha⁻¹ during 2016 and 2018.

On the Agronomy Farm in Auburn, the field was disked and beds were constructed with a tractor drawn bedder (Nolts). Three trials were located in different positions in the same field on the Agronomy Farm. The field was disked on May 28, 2016, June 1st, 2017 and May 12th, 2018. Bed distance was 170 cm, width of bed was 66 cm, 216 cm long.

The distance between adjacent beds was 104 cm. Drip tape (Toro Aqua-Traxx 0.45 GPM) was applied down the center of each row. Beds were constructed with tractor drawn after disking. The fields were mulched with wheat straw prior to transplanting. Over three years, plots received three supplemental fertilizer applications (using 4-3-2 Miracle-Gro Organic) throughout the growing season. Supplemental fertilizer applications were applied in July, September, and October and provided an additional 9 kg N ha⁻¹, 12 kg P₂O₅ ha⁻¹ and 6 kg of K₂O ha⁻¹ each time.

Experimental Design

A randomization complete block design was used with four replications. The number of plots in each replication was based on how many varieties involved every year.

In 2016, there were eight varieties. In the field, plots within replications were oriented in parallel from east to west; replications and rows were oriented south and north. Each replication contained 8 experimental plots from west to east. There was one border plot each side of the trial. The varieties CA1, CL2, CL3, CL5, CL6, CL7, CL8 and CL9 were assigned randomly in each replication. The target width was 0.9 meter but because of the bedding equipment used the actual spacing was 1.8 meters between rows. CL10 which was not available in sufficient quantity in 2016 was added to the trial in 2017. The varieties included in 2017 were selected based upon dry yield and curcumin yield, and planted it in 2017 with same experimental design, i.e. randomization complete block design. The process was repeated in 2018.

Transplanting and Field Operations

Turmeric seedlings were transplanted into field on June 7th, 2016; May 26th, 2017; June 7th, 2018. Plants were laid out by hand at each site. Seven plants were planted per row. Sprouted rhizomes were allocated into plots in such a way as to ensure that each plot of a given variety received plants of similar size. The plants were spaced 30 cm in rows.

Immediately following planting, the plots were irrigated with overhead sprinklers. Further irrigation was done with drip. The plots were hand weeded throughout the summer and fall. Sandea (Halosulfuron-methyl, methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl) and 1-methylpyrazole-4-carboxylate, Gowan Co., Yuma, AZ) were applied between the rows to control purple nutsedge in 2018. A single application of Finale (glufosinate-ammonium; Bayer CropScience) was applied around the perimeter of the field to control broadleaf weed species in August during the three years. Hand weeding was carried out in the rows to prevent crop damage.

Plots received three supplemental fertilizer applications (using 4-3-2 Miracle-Gro Organic) throughout the growing season in 2016. Supplemental fertilizer applications were applied in July, September, and October and provided an additional 9 kg of N, 12.3 kg of P and 6.2 kg of K per hectare. Liquid fertilization (20-10-20) was applied in 2017 and 2018 to replace Miracle-Gro. Foliar Maxicrop liquid seaweed was applied one month after transplanting (Ohrstrom's, USA). During growth and development periods, field checks were done weekly, to protect the plants from weeds, disease and pests.

Harvest and Post-harvest Handling

Matured turmeric rhizomes were harvested on January 23, 2017; January 9, 2018; January 23, 2019. Normally we harvested turmeric rhizomes the year following planting, i.e. 2016 field trials were harvested in 2017, 2017 trials were harvested in 2018. Shears were used to cut the aboveground turmeric shoots. Digging forks and shovels were used to dig rhizomes out of ground, being careful to keep a little distance from turmeric plant to avoid damaging rhizomes. There were seven plants each plot, but we harvested the middle five plants for yield determination and analysis. Rhizomes from each plot were placed into marked or labeled containers to avoid mixing different plot rhizomes together.

Harvested turmeric rhizomes were broken apart and washed with sprayer to remove soil and other unwanted material trapped between the rhizomes. Washed turmeric rhizomes were spread on Kraft paper to surface dry. Fans are used to help accelerate this process. Mother rhizomes, finger rhizomes, frozen rhizomes and diseased rhizomes were sorted and weighed separately.

Representative subsamples of mother and finger rhizomes from each plot were weighed and stored separately. For each plot, we collected around 400 g finger rhizome, 350 g mother rhizome as subsamples for drying. These samples were weighed and placed in open paper bags until such time that the samples could be completely dried down in a forced air dryer (Batch) at Alabama Seed Technology Center. The dryer was set at 43 °C. Samples were kept in the dryer until weights stabilized for two consecutive days without

losing further moisture which was assumed to be 0 % moisture. Once weights stabilized, samples were stored in Ziploc plastic bags.

Data Analysis

Data was analyzed using R 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria), Excel 2019 (Microsoft Excel. Retrieved from <https://office.microsoft.com/excel>) and SPSS 26.0 (IBM Corp., Armonk, N.Y., USA). These were used for calculated and analyzed mean, standard deviation, coefficient of variation, Shapiro-Wilk normality test and Bartlett test of homogeneity of variances. Coefficient of variation (CV) was used to show the degree of dispersion of finger rhizome, mother rhizome and total performance. One-way and two-way ANOVA tests included 0.05 and 0.1 significance levels. Tukey test was used to detect difference within groups. There were some missing data in 2016 and 2018, used linear model, mice function in R to fill the missed data. The following equation shows the calculation of CV value.

Equation 1:

$$C.V = \frac{\sigma}{\bar{y}} * 100\%$$

σ = Standard Deviation

\bar{y} = Average Yield

Linear model and mice function were used to estimate missing data. In 2016, we estimated values for two missing plots of CL9. For fresh rhizome yield, we estimated

values of 18945 for replication 3, 23641 for replication 4. The total rhizome percent dry weight of CL9 was 18.4 %, hence, the dry rhizome yield of CL9 at replication 3 and 4 will be 3486 and 4350. Missing CL9 plots were replaced by CL8, all 6 plots of CL8 were remained for further analysis.

Table 7 gives the results of Shapiro-Wilk and Bartlett test results of fresh rhizome yield, rhizome dry weight yield and percent dry weight of rhizome in 2016, 2017 and 2018, respectively. Test results indicated that the rhizome yield data in three years has normality. And Bartlett test showed that variances of each group did not differ and are equal.

Covariate Analysis

Over three years, diseased rhizome yield and percentage of weight of diseased rhizome showed huge difference within plots and years. Especially in 2018, the average percent of diseased rhizome weight was 16.2 %, but the disease percentage for CL3 was 37.3 %, much higher than for CA1 and CL9. The worst disease appeared at the southeast corner, especially in plots 13, 14, 24 and 34. And field observation also showed plants in these plots looked smaller and less vigorous than others. CL3 in plot 14 had 100 % of diseased rhizomes. Diseased rhizomes were included with healthy rhizomes during following analysis. Heavy disease could cause enormous yield loss, and this could affect the yield comparison because all varieties were not affected equally. To reduce this potential negative effect, we used the percentage weight of diseased rhizome as the

covariate and used covariate test analyze the fresh rhizome yield, rhizome dry weight yield and curcumin yield in 2018, which had the worst disease.

Tests were conducted to verify if covariate analysis could be applied to the turmeric data. As for total fresh rhizome yield, the Shapiro-Wilk test was used on the original data to analyze the residuals of each variety, to determine if residuals have normality. P values of result of CA1, CL3, CL9 and CL10 were 0.390, 0.908, 0.068 and 0.340. All of them were higher than 0.05, confirming normality. For Levene's test of equality of error variances, the p value was 0.336, which was higher than 0.05. Equality of error variance was confirmed. The next step was to check standardized residuals list of fresh rhizome yield. There was no number higher than 3, signifying that there were no outliers. The test of between-subjects' effects gave a p value for Variety of 0.048. That means there were significant differences between turmeric varieties at 0.05 and 0.1 confidence levels. Tukey HSD was used to do the post hoc test. Only the groups of CL9 and CL3 had significant difference at 0.1 confidence level, which p value was 0.091. Based on these test results, turmeric fresh rhizome yield data in 2018 met all requirements of covariate test.

As for the rhizome dry weight yield, the Shapiro-Wilk test was used to analyze the residuals of each variety, if residuals have normality. P values of result of CA1, CL3, CL9 and CL10 were 0.110, 0.980, 0.067 and 0.855. All of them were higher than 0.05, normality was confirmed. Eight residuals of dependent variable had equal variance. For

Levene's test of equality of error variances, the p value was 0.783, which was higher than 0.05. Equality of error variance was confirmed. The next step was to check standardized residuals list of rhizome dry weight yield. There was no number higher than 3, meaning that there was no outlier. Test of between-subjects effects shows, p value of variety was 0.301. That means there was no significant difference within varieties at 0.05 and 0.1 confidence level. Based on these test results, turmeric dry weight yield data in 2018 met all requirements of covariate test. And then, covariate test was used to analysis 2018 turmeric rhizome yield data.

Rhizome Yield Performance Across Years

Yield performance is the key point for a crop variety, but a successful variety should not be limited to high yield, how steady of its annual performance could decide how far could it go in the future. A good and successful variety normally should have good and steady yield performance. Hence, after the yield performance analysis, next step is to move on the part of stability. Three methods were used to test the stability of varieties over three years, including coefficient variation (CV), stability parameter (ai) and high stability coefficient (HSC).

These analyses were applied to fresh rhizome yield and rhizome dry weight yield. Stability rank may vary from different test methods. Results of the three methods were compared.

The ai and CV test results were used to draw four-quadrant diagrams. The vertical

axis is rhizome or curcumin yield, and the horizontal axis represents the a_i or CV value. Variety in the first quartile means it has high yield but low stability; variety in the second quartile means it has both high yield and high stability; variety in the third quadrant means it has low yield but high stability; variety in the fourth quadrant means it has both low yield and low stability. Varieties having an a_i value closer to 1.0 or equal to 1.0, is likely suited for more different environments; if the a_i value is much lower than 1, it has acceptable stability but low potential and cannot utilize beneficial environmental conditions; if the a_i value is much higher than one, it means that it has unsatisfied stability performance, is not good as others, and is sensitive to environmental conditions i.e. has outstanding performance with good conditions, but yield is reduced a lot when the variety meets adverse conditions (Bingwei et al., 2008).

The HSC, is simpler to interpret than a_i and CV. A lower HSC value means better yield and stability performance. These equations used to obtain the three measures of stability are given below.

Equation 1:

$$C.V = \frac{\sigma}{\bar{y}} * 100\%$$

σ = Standard Deviation

\bar{y} = Average Yield

Equation 2:

$$a_i = \frac{S_i}{S}$$

S_i =Standard Deviation of Each Variety

S =Standard Deviation of All Varieties

Equation 3:

$$HSC = \left(\frac{1 - (y_i - s_i)}{1.10\bar{y}} \right) * 100\%$$

y_i =Yield or Concentration of Each Variety

\bar{y} = Average yield or concentration of All Varieties

S_i =Standard Deviation of Each Variety

Disease Assessment

According to normal field checks over three years, some plots showed growth problems. Like plot 14 and 34 in 2018 trial, some of plants in these plots look obviously weaker than other plants and other plots. Which have shorter plant heights and smaller leaves. Plot 35 in 2017 had severe wilt problems, and other leaves had a lot of big spots on leaves. Besides, each year after harvest, many plots had high percentage of abnormal rhizomes. These rhizomes had slight to severe decay, soft texture. And the color of these rhizomes was different from normal color, water stained and brown. A foul smell always could be noticed from them. These symptoms were similar to traits of common turmeric diseases, like rhizome rot and leaf spot. Hence, some investigations were conducted to find possible reason and or pathogens. Each year after harvest, diseased rhizomes were separated from healthy rhizomes, weighed and bagged. Collected data was used to

calculate the average disease percentage of varieties every year. The F test was used to determine the difference between varieties. The average disease percentage was determined annually. Nematodes are common pathogens of turmeric, so they were highly suspected pathogen.

a. Turmeric Nematode Assessment

To investigate the nematode pathogens in the turmeric field trials, soil samples were taken for each plot on January 14th, 2019 at Auburn experimental field. Soil samples were taken close to turmeric rhizomes. A separate soil sample was collected from each turmeric plant. The soil was kept in plastic food bags, the volume of soil samples over half bag. Each sample bag and saved in refrigerator around 38 °C. A plastic cup was used to measure 250 ml soil to provide uniform volumes of soil. The soil sample was poured into a bucket, into which was added purified water to make soil suspension. Two sieves were used to filter soil-water mixture. The bottom sieve measured 0.0017-inch, 45 micrometers, while the top sieve measured 0.0029-inch, 75 micrometers. The residue on the top of bottom sieve was transferred into clean cups. The solution used for flush was limited to the quarter volume of cup.

The residue samples were poured into marked culture tubes. A solution of 454 g cane sugar L⁻¹ was prepared and added to fill each tube. The samples were then centrifuged for 4 minutes at 2000 r·min⁻¹. The filtrate was then sieved through two sieves measuring 0.0029-inch, 75 micrometers at the top and 0.0010 inch, 25 micrometers at the

bottom.

A microscope was used to search and observe nematodes in final samples. A few drops of hydrochloric acid solution were added to restrain the motility of nematodes. Nematodes were identified roughly by the stylet. More attention was given to nematodes with stylets, which were likely plant-parasitic nematode. This was carried out on January 17th, 2019 At the Plant Pathology nematode lab.

Data was collected from AWIS Weather Service, Inc. (<https://www.awis.com/mesonet/>). including monthly active accumulated temperature, solar radiation, precipitation, evaporation, rain day, humidity, and chill hour during May to next year's January.

Result and Discussion

In 2016, finger and mother rhizome yields were almost the same, with respect to both fresh and dry rhizome yields. For example, the fresh finger rhizome yield and mother rhizome yield in 2016 were 11370 kg ha⁻¹ and 11340 kg ha⁻¹, respectively. But in the next two years, finger rhizome had higher rhizome yield, even doubled. The fresh finger rhizome and mother rhizome yields in 2017 were 8310 kg·ha⁻¹ and 4250 kg·ha⁻¹, respectively. A likely reason is that finger rhizomes can turn into new mother rhizomes, having shoots and roots. A mature mother rhizome is very distinct in appearance from a finger rhizome, but a new mother looks very much like a finger rhizome, which is once was. This introduces an element of subjectivity in deciding whether to classify some

rhizomes as mothers or fingers. In the 2016 trial, stricter definition of mother rhizomes was applied based upon presence of shoots than was applied in subsequent years.

2016-Fresh Rhizome Yield

Average fresh finger rhizome, fresh mother rhizome and total (finger plus mother) fresh rhizome yields of eight varieties were 11370 kg·ha⁻¹, 11340 kg·ha⁻¹ and 22670 kg·ha⁻¹, respectively (Table 8 and Figure 3). CL2 had the highest fresh finger rhizome, fresh mother rhizome and total fresh rhizome yields, which was almost 50 % higher than the average yields of all varieties. There were no significant differences in fresh finger rhizome and total fresh rhizome yield at significance levels of 0.05 and 0.1. Fresh mother rhizome yields had significant differences at a significance level 0.1. The P value for replications was 0.343, which meant that the environmental conditions of different blocks did not affect these characters significantly.

2016-Rhizome Dry Weight Yield

The dry finger rhizome, dry mother rhizome and total dry rhizome yields of the eight varieties were 1820 kg·ha⁻¹, 2250 kg·ha⁻¹ and 4070 kg·ha⁻¹ (Table 9 and Figure 4). CL2 had the highest dry finger rhizome, dry mother rhizome and average dry rhizome yields, which was almost 50 % higher than the average performances. In 2016, there were significant differences on dry finger rhizome, dry mother rhizome and total dry rhizome yield at significance levels of 0.05 and 0.1. CL2 had significantly higher finger, mother, and total dry weight yields than other varieties except CL7. CL6 had the lowest yields. P

value of replications was 0.156, which meant the environmental conditions of different blocks did not affect these characters significantly.

2016-Percent Dry Weight of Rhizome

Percent dry weight of finger, mother, and weighted average (finger plus mother) percent dry weight total rhizome of the eight varieties were 15.7 %, 19.7 % and 17.7 %, respectively (Table 10). There were significant differences in percent dry weight of finger, mother, and total rhizomes at significance level 0.05 and 0.1. CL2 had significantly higher percent dry weight of finger, mother and total rhizome yields than other varieties except CL7 for finger, mother and total rhizomes. CL5 and CL6 had the lowest percent dry weight compared to other varieties. Environmental conditions of different blocks did not affect these characters significantly.

2017-Fresh Rhizome Yield

CL2 and CL7 were dropped from 2017 trial and CL10 was added. In 2017, average fresh finger, mother and total rhizome yields of seven varieties were 8310 kg·ha⁻¹, 4250 kg·ha⁻¹ and 12580 kg·ha⁻¹, respectively (Table 11 and Figure 5). CA1 had the highest fresh finger rhizome, fresh mother rhizome and average fresh rhizome yields. The seven varieties included in the trial did not differ significantly for fresh finger rhizome, fresh mother rhizome and total fresh rhizome yield at significance level 0.05. However, there were significant differences for fresh finger rhizome yields at significance level 0.1. CA1 had the highest total fresh rhizome yield. The P value for replications was not significant,

which meant that the environmental conditions of different blocks did not affect these characters significantly. CL9 had very high fresh rhizome yield in 2016, but in 2017 its fresh yield was lower than the average performance. The reason for the poorer performance of CL9 in 2017 than in 2016 is not very clear. The new variety, CL10, had good performance.

2017-Rhizome Dry Weight Yield

Average finger mother and total dry rhizome yields in 2017 were 1430 kg·ha⁻¹, 880 kg·ha⁻¹ and 2310 kg·ha⁻¹, respectively (Table 12 and Figure 6). CA1 had the highest dry finger rhizome, dry mother rhizome and total dry rhizome yields. Differences were significant for finger and total dry rhizome yields at significance level 0.05 and 0.1, respectively, but there was no significant difference for dry mother rhizome yield. CA1 had the highest rhizome dry weight yield and it was significantly different from CL5, CL6 and CL9. P value of replications was 0.713, that meant the environmental conditions of different blocks did not affect these characters significantly. The new variety, CL10, had good performance on this.

2017-Percent Dry Weight of Rhizome

In 2017, average percent dry weight of finger, mother, and total rhizomes for the seven varieties were 17.4%, 20.7% and 18.5%, respectively (Table 13). CL10 had the highest percent dry weight of finger, mother, and total rhizome yields. Difference were significant at the 0.05 level of significance for percent dry weight of finger, mother, and

total rhizomes. CL10 was significantly different from other varieties, except CL8. And its finger percent dry weight was only different from CL5, CL6 and CL9. Blocks did not test significant.

2018-Fresh Rhizome Yield

In 2018, average fresh finger, mother and total rhizome yields were 18520 kg·ha⁻¹, 10080 kg·ha⁻¹ and 28400 kg·ha⁻¹, respectively (Table 14, Figures 7 and 8). Differences for fresh finger, mother and total rhizome yield were significant at the 0.05 level. CA1 had the highest fresh finger rhizome, it is significantly different from CL3 and CL10. CL9 had the highest fresh mother rhizome and average fresh rhizome yields, and it is significantly different from CL3. P value of replications was 0.227, which means the environmental conditions of different blocks do not affect these characters significantly.

2018-Rhizome Dry Weight Yield

Dry finger rhizome, mother and total rhizome yields in the trial averaged 3020 kg·ha⁻¹, 1950 kg·ha⁻¹ and 4970 kg·ha⁻¹, respectively (Table 15, Figure 9). CA1 had the highest dry finger rhizome and average dry rhizome yields. CL10 has the highest mother dry weight yield and it was significantly higher than CL3. However, there were no significant differences in finger and total dry rhizome yield at significance level 0.05. CA1 and CL9 were had significantly higher finger and total dry weight yield than CL3 and CL10 at significance level of 0.1 The rankings were same for finger and total dry weight. CL3 was significantly lower than other three varieties at significance level of

0.05. The P value of replications was 0.219, that meant the environmental conditions of different blocks did not affect these characters significantly.

2018-Percent Dry Weight of Rhizome

The percent dry weights of finger, mother, and total rhizomes of the four varieties tested in 2018 were 16.8 %, 19.9 % and 18.0%, respectively (Table 16). Significant differences were observed in percent dry weight of finger rhizome, mother rhizome and total rhizome. CL10 had the highest percent dry weight and it was significantly different from CA1 and CL9, but it only significantly different from CL9 for mother rhizome percent dry weight. Blocks did not test significant.

Covariate Analysis of Rhizome Yields

Diseased fresh rhizome weight and percent of diseased fresh rhizome weight were analyzed over three years (Table 29, 30 and 31). Results showed that there was no significant difference within varieties for both diseased rhizome weight and percent of diseased rhizome weight. Years had significant effect on diseased rhizome weight but not for percent of diseased rhizome weight. Interaction of variety and year had no significant difference on these two characters. To reduce the effects of disease on rhizome yield, especially in 2018, percentage of disease was 16.2%. Therefore, covariate test was used to help detect the real difference of rhizome yield potential.

Comparing the covariate test result with the original test result, covariate test reduced the differences between varieties (Tables 14 and Table 15). The rank of yield

from high to low did not change based on covariate test, narrower gaps show between the high yield and low yield data. And there was no new appeared significant difference within varieties. Results were almost same. CL9 had the highest fresh rhizome yield. CA1 had the highest rhizome dry weight yield. But the varieties were not significantly different from each other.

The lack of differences following covariate analysis can be explained by the fact that, the four most heavily diseased plots, 13, 14, 24 and 34 all belonged to two varieties, CL3 and CL10, which were the lowest yielding varieties. Without the effect of disease, CL3 and CL10 would have performed better, and difference among varieties should have been smaller.

Rhizome Yield Performance across Years

The performance of the four varieties retained in 2018, CA1, CL3, CL9 and CL10, were compared over the three years of the trials (Figure 10 and Figure 13). The CV% method (Figure 11) shows that CL3 had the best stability on total fresh rhizome yield, followed by CA1, CL10 and CL9. But the yield of CL3 was lower than mean. CA1 had the highest yield and acceptable stability. The ai method shows that CL10 had the best stability, followed by CL3, CA1 and CL9 (Figure 12). Table 17 shows that CA1 had the best stability, followed by CL3, CL10 and CL9 based upon the HSC test result.

Summarizing these three test results, CL9 had the worst stability in all three methods because it performed poorly in 2017, but in other two years it performed fairly well. It

appears sensitive to environmental conditions, had high rhizome yield with suitable conditions, very low yield when conditions were not satisfied. Summarizing these three ranks, CL3 had the best stability followed by CA1 and CL10, which implies that they were less sensitive to environmental conditions, but CL3 cannot utilize the beneficial conditions very well. In other word, CL3 had low potential on yield.

For rhizome dry weight yield, the CV% test result shows that CL3 had the best stability, followed by CA1, CL10 and CL9 (Figure 14). But its rhizome yield was lower than mean. CA1 has the highest yield and acceptable stability. Ai test result (Figure 15) shows same result as CV%. Table 18 shows, CA1 had the best performance on stability and yield as determined by the HSC test, followed by CL9, CL3 and CL10.

Comparing three stability test results and summary, CL9 had lower stability than the other three varieties. CL3 had the best stability followed by CA1 and CL10.

Disease Fluctuation

Diseased fresh rhizome weight and percent of diseased fresh rhizome weight were analyzed over three years. Results showed that there was no significant difference within varieties on both diseased rhizome weight and percent of diseased rhizome weight. Years had significant effect on diseased rhizome weight but not for percent of diseased rhizome weight. Interaction of variety and year had no significant difference on these two characters. As for performance of each year and replications. Significant difference appeared on diseased rhizome weight within replications in 2016. Eight varieties in 2016

had significant difference on percent of diseased rhizome weight, also appeared on replications. But as for CA1, CL3, CL9, there was no difference. Overall, four varieties finally selected, CA1, CL3, CL9 and CL10, no variety was easier infected than others. Due to some unclear environmental conditions, the highest diseased rhizome weight and percent of diseased rhizome weight were observed in 2018, the lowest data were observed in 2017.

Between May and January is the period during which turmeric plants stay in field. From May to October, this is the growing season, climatic conditions in these months could affect the yield of rhizome and curcumin. The last three months, November, December and January, are the maturation stage of turmeric rhizomes. Turmeric stops growing, transfers nutrients to underground part. It is sensitive to pathogens and extreme climatic conditions and these could make it worse.

Even some varieties showed obvious high or low disease percentage (Table 29, 30 and 31), but move ahead to every plot performance, we found that the mean number of each variety cannot truly show the real disease performance of varieties, like in 2018, almost all CL3 plants in replication 1 was diseased, but in replication 4, its disease percentage just around 3%. According to this kind of example, even the F test shows some varieties had significant difference with others, but the likely reason was about the location of plot, not the varieties themselves.

Based on above study result, many characters showed big difference over three years

(Table 29, 30 and 31). Planting locations were almost same and other controllable treatments like fertilizer were also same. There were probably some climatic characters that affected the rhizome yields and disease.

Auburn has a humid subtropical climate, which consists of early spring, long and hot summer, warm fall, and mild winter. Abundant rainfall is concentrated on spring and summer. May to October is regarded as the plant growing season. November to January is regarded as the rhizome maturation season. Climatic data in January of each year was only collected for the days before harvest. Figure 16, 17 and 18 show the main climatic characters in local area, monthly active accumulated temperature, precipitation, and solar radiation.

Monthly active accumulated temperature data were almost the same over years, but temperatures for 2017 were always slightly lower than other two years (Figure 16). No extreme weather was recorded in growing months during three years, like frost and extreme high temperature. Hence, temperature likely is not the main point which brings remarkable fluctuation on yields over years.

But precipitation was different. Accumulated precipitation between May to January was 63.4 cm, 96.4 cm and 113.2 cm for the three years (Figure 17), respectively. During the plant growing season, precipitation in 2018 was more stable than other two years; 2017 had the highest precipitation in the first 3 months in field, and it brought standing water problems in field in June. 2016 had a little rainfall in the first several months of the

growing season. Overall, uniform and abundant precipitation may contribute to the highest rhizome and curcumin yields, especially excessive rainfall happened during a short period. Precipitation in the first and last months in 2017 may bring too much water and get the lowest yield performance. 2016 had the middle performance (Figure 17).

During the rhizome maturation period, 2017 had the lowest precipitation; precipitations in 2016 and 2018 were much higher. This may explain why 2017 had the lowest percent of diseased rhizome weight: 3.3% compared to 10.9 % and 16.2 % in the other two years. In the months before harvest in 2016 and 2018, precipitations were more frequent and heavier than in 2017.

Daily solar radiations over three years were almost same in the three years, except for some days in June 2017, its solar radiation was obvious lower than other two months in 2016 and 2017 (Figure 18).

Chill hour data during growing season within these three years were obtained from AWIS Weather Service website. Too much chill weather may bring huge yield loss and or much disease. 2017 had the most chill hours, 719, compared to 435 and 537 in 2016 and 2018. In addition, first frost appears on November 20th, 2016, October 28th, 2017 and November 16th, 2018. Frost came over a half month earlier in 2017 than in the other two years. This shortened the growing season, and may have contributed to lower yields together with the highest chill hours.

Turmeric Nematode Assessment

Figure 25 shows some nematodes got from soil samples. No nematode with stylet found within observation, which means likely no plant-parasitic nematode in this investigation.

Conclusion

In all, CL2 had the highest fresh and dry rhizome yields, CL7 also had a very good rhizome yield. CL2 had 53.1 % and 62.2 % higher fresh and dry rhizome yields than the mean of the trial. It yielded significantly higher than other varieties except CL7. Both varieties also had high rhizome percent dry weight. But based on some curcumin content reported in chapter 3, CL2 and CL7 have significantly lower curcumin concentration and curcumin yield. Since the desired turmeric varieties must also have acceptable curcumin content, during 2016 and 2018 field tests showed CA1 was a good variety with very high fresh and dry rhizome yield, and it also has good performance on curcumin concentration and yield.

CL5 had high fresh rhizome yield, but percent of dry weight of total rhizome and dry rhizome yield were both close to mean. CL3 had the best stability on fresh rhizome yield and rhizome dry weight yield but low yield. CL9 has high rhizome yield but it appears to be very sensitive to environments and was not stable over years. CL10 was not outstanding on either rhizome yield or stability.

Of the four varieties retained over the three years, CA1 had the highest fresh and dry rhizome yields with acceptable stability, slightly sensitive to environmental conditions. It

is one of strongly recommended variety for Alabama based on good yield and stability performances. If fresh and or dried rhizome yields is the main objective without regard to curcumin content, CL2 and CL7 could be optional varieties, but their stability should be tested in different years and locations. CL9 also is a good variety on rhizome yield and curcumin, but its rhizome yield appears sensitive to environmental conditions. Therefore, in some desirable environments, CL9 could be considered as a choice and may get high rhizome yield, but also needs more tests to verify. CL9 had the worst performance in 2017, however, its plots were mostly located on the east side of the field, which showed heavier disease compared to west side. Hence, CL9 had the worst stability by the CV and ai methods, in 2017 three of four plots were located in a side of the field that had poorer growth, may have better stability than what was calculated. In other word, CL9 may have better stability than what was calculated.

Slightly difference in common climatic conditions, like active accumulated temperature, solar radiation, precipitation, evaporation, rain day, humidity and chill hour likely did not affect turmeric rhizome and curcumin performances. There may be some other characters of soil or cultivation contributes to huge difference in three years. Precipitation difference in last two or three growing months of turmeric could affect disease conditions closely.

Fresh and dried rhizome yields showed huge difference fluctuation over years. It will be useful to carry out research to find out what environmental influence contributes

to these fluctuations in the future and whether there are any treatments that we could apply to reduce the negative effects and improve the performance.

Chapter 3 Curcumin Concentration and Yield

Introduction

Turmeric (*Curcuma longa* L.) is a rhizomatous herbaceous perennial plant and belonging to the ginger family, *Zingiberaceae*. Currently, more than 80% of turmeric is produced by India and turmeric products are sent to numerous countries. China also exports turmeric to supply this demand (Wickenberg et al., 2010), but India still is the biggest consumer, producer and exporter of turmeric over the world. India planted almost 173 thousand hectares of turmeric during the year 2005-2006 (Bansal et al., 2008). In ancient India, turmeric was regarded as a gift from nature, this magic plant provided people food and health. Now with the development of modern chemical analysis method, turmeric is facing more opportunities in new areas, like pharmacy.

Intact dried rhizomes and its extracts, turmeric powder (ground turmeric), curcumin, oleoresin and volatile oil are the main ingredients used for medical purposes (Gounder and Lingamallu, 2012). Many diseases like Alzheimer's diseases, diabetes, cirrhosis and colitis are caused by inflammation. Adding some components of turmeric into medicine can relieve the inflammation and treat these diseases (Olojede et al., 2009).

Pure curcumin is a yellowish to orange red crystalline powder, whose chemical formula is $C_{21}H_{20}O_6$. Its molecular weight is 368.38, and it is insoluble in water (Bansal et al., 2008). Curcumin is isolated from ground rhizomes of turmeric as a yellow pigment.

The curcumin content varies greatly among varieties, planting locations, sources, and cultivation conditions. Average curcumin concentration of turmeric powder is around 4% by dry weight (Li et al., 2011).

Curcumin consists three curcuminoids: curcumin I (diferuloylmethane, 94%, $C_{21}H_{20}O_6$, $368.4 \text{ g}\cdot\text{mol}^{-1}$), curcumin II (demethoxycurcumin, 6%, $C_{19}H_{16}O_4$, $308.3 \text{ g}\cdot\text{mol}^{-1}$) and curcumin III (bisdemethoxycurcumin, 0.3%, $C_{20}H_{18}O_5$, $338.4 \text{ g}\cdot\text{mol}^{-1}$) (Bansal et al., 2008; Prasad and Bgarwal, 2011).

Curcumin has anti-inflammatory, hypolipidemic and antioxidant properties as does vitamins C, E and Beta-Carotene, giving it the potential to be considered in the development of cancer preventive strategies, liver protection and applications in clinical research. Based on animals and human clinical tests, it has been proven that curcumin is safe even at high doses (Grewal et al., 2003). There are multiple pathways by which curcumin is effective against inflammation. First, curcumin decreases the production of inflammatory substances. Secondly, it can enhance or extend the reaction of the body toward inflammation, specifically, it increases the secretion of adrenal hormone and cortisol. Third, curcumin can promote circulation of toxic substances which accelerate spitting out of them from inflamed tissues (Mishra and Palanivelu, 2008). In this project, HPLC was used to analyze curcumin, and its testing conditions were list in Table 4.

Recent studies have shown that curcumin may contribute to fight against cancers. Tumor cells use multiple pathways to protect them from the attack of host's immune

system, but scientists have found curcumin can inhibit signal conversion pathway of tumor cells. In this case tumor cells become fragile and can be removed easily.

Turmeric's other property, anti-angiogenesis is also useful against tumors (Ravindran et al., 2007). In the future curcumin may save cancer patients.

With the increasing demand of turmeric and curcumin on food and pharmacy, American has been the biggest importer of turmeric over the world, around 29% of global turmeric market. And its import value reached 381 million dollars in 2017, and keep increasing (Nguyen et al., 2018). In this case, anticipation is growing to start cultivation of good turmeric varieties with high curcumin yield and other desirable performances. Specially, turmeric has potential as a high value crop for local area, Alabama. It is a new crop and information is lacking on the best varieties to grow.

Hence, our research objectives were to select high curcumin concentration and high curcumin yield turmeric varieties. The primary target was curcumin yield.

Materials and Methods

There was a three-year process of elimination to arrive at the varieties that best met the requirements of the trial. The origin of the varieties is listed in Table 5. Field trials were conducted at Auburn Agronomy Farm (32° 36' 35.4816" N, 85° 28' 50.8152" W) during 2016, 2017 and 2018. Soil was tested prior to fertilization in each season. Soil tests result given in Table 6, pH values are range from 5.7 to 6.1, acid soil, loams and light clays soil type. These were randomized complete block trials with for replications,

and detailed experimental design and trial practices were stated in chapter 2.

Each year, after harvesting turmeric, healthy turmeric rhizomes were separated into finger and mother rhizomes. Representative subsamples of mother and finger rhizomes from each plot were weighed and stored separately. For each plot, we collected around 400 g finger rhizome, 350 g mother rhizome as subsamples for drying. These samples were weighed and placed in open paper bags until such time that the samples could be completely dried down in a forced air dryer at Alabama Seed Technology Center. The dryer was set at 43°C. Samples were kept in the dryer until weights stabilized for two consecutive days without losing further moisture which was assumed to be 0 % moisture. Once weights stabilized, samples were stored in Ziploc plastic bags.

Based on this, the weight of tested turmeric rhizome samples was used to calculate the final curcumin yield of turmeric varieties every year. Statistics were used to test if there were any differences of curcumin concentration and yield within varieties. In addition, three methods were used to test the stability of curcumin for each variety, ai, HSC and CV %.

Analysis of Curcumin Concentration

Curcumin determinations were carried out at Alabama A&M University during 2017, 2018 and 2019 following harvest of trials from preceding year. The HPLC method was used to analyze the three kinds of curcumin concentration in dried turmeric rhizomes. HPLC gave the separately contents of curcumin I, II and III, these three parts

were combined to get the total curcumin concentration we need. Curcuminoids were extracted from ground turmeric samples, using 70% ethanol at room temperature for 18 h of shaking. A high vacuum evaporator was used to concentrate the residue then. The HPLC analysis was performed using an Agilent 1100 series HPLC system equipped with an Agilent Plus C₁₈ column 5mm (150 x 4.6 mm); isocratic elution used acetonitrile and 2% acetic acid; 60%^B, flow rate was 2 mL·min⁻¹; column temperature was set at 33 °C; UV detection was 425 nm; curcuminoid standard calibration curves were used to quantify the curcuminoid contents in turmeric extracts; curcuminoid content in extracts was calculated based upon dry weight of turmeric samples (mg·g⁻¹). Solvent material weight was 10:1.

Statistical analyses were also performed to detect varietal differences in individual curcuminoids (curcumin I, II and III + curcumin I, II and III). The curcuminoid data from 2016 were used for this analysis except for CL10 which was obtained from 2017 due to its absence in first year of the trials.

Data Analysis

Data were subjected to R 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria), Excel 2019 (Microsoft Excel. Retrieved from <https://office.microsoft.com/excel>) and SPSS 26.0 (IBM Corp., Armonk, N.Y., USA). This software was used to calculate and analyze mean, standard deviation, coefficient of variation, Shapiro-Wilk normality test and Bartlett test of homogeneity of variances.

Coefficient of variation (CV) was used to show the degree of dispersion of finger rhizome, mother rhizome and total performance. One-way and two-way ANOVA tests were used to analyze the difference within varieties, which included 0.05 and 0.1 significance levels. The Tukey test was used to detect differences within groups. There were some missing data in 2016 and 2018. In 2016, because of the shortage of planting materials, CL9 was just planted in replications 1 and 2. In 2018, plants in the plot of CL3 in replication 1 were all diseased, so it was removed for future curcumin analysis and testing. These needed to be filled before analysis. Linear model and mice function were used to estimate some absent values, CL9 curcumin yield data in 2016 (231.4 for replication 3, 87.7 for replication 4), and CL3 curcumin yield data in 2018 (125.9 for replication 1).

Table 19 gives the results of Shapiro-Wilk and Bartlett test results of curcumin concentration and curcumin yield of turmeric in 2016, 2017 and 2018. Test results indicated that the curcumin data in three years has normality. And Bartlett test showed that variances of each group did not differ and are equal.

Covariate Test

In 2018, the average percent of diseased rhizome weight was 16.2%. Variety CL3 had a disease percentage is 37.3%, much higher than CA1 and CL9. The worst disease appeared in the southeast corner, especially at plot 13, 14, 24 and 34. Variety CL3 in plot 14 had 100 % diseased rhizomes. To reduce this potential negative effect, the percentage

of weight of diseased rhizome was set as the covariate, and covariate test was used to analyze the curcumin concentration and curcumin yield in 2018, which has the worst disease. Covariate test was conducted directly based on original unadjusted yield data, adjusted curcumin data was used to compare and analysis.

Curcumin Yield Stability Analysis

Three methods were used to test the stability of total curcumin yield of varieties over three years. These methods included coefficient variation (CV), stability parameter (ai) and high stability coefficient (HSC). Stability rank may vary from different test methods. Results of three methods were compared. The following equations show how the three stability test methods are calculated.

Equation 1:

$$C.V = \frac{\sigma}{\bar{y}} * 100\%$$

σ = Standard Deviation

\bar{y} = Average Yield

Equation 2:

$$ai = \frac{S_i}{S}$$

S_i =Standard Deviation of Each Variety

S =Standard Deviation of All Varieties

Equation 3:

$$HSC = \left(\frac{1 - (y_i - s_i)}{1.10\bar{y}} \right) * 100\%$$

y_i =Yield or Concentration of Each Variety

\bar{y} = Average yield or concentration of All Varieties

S_i =Standard Deviation of Each Variety

A_i and CV test results were used to draw four-quadrant diagrams. The vertical axis is curcumin yield, and the horizontal axis represents the a_i or CV value. If a variety is in the first quartile, it has high yield but low stability; a variety in the second quartile has both high yield and high stability; a variety in the third quadrant has low yield but high stability; a variety in the fourth quadrant has both low yield and low stability. Other explanations for a_i result is that the value closer to 1.0 or equal to 1.0, means it likely suit for more different environments; if a_i value is much lower than 1, that means it has acceptable stability but low potential and cannot utilize beneficial environmental conditions; if a_i value is much higher than 1, that means is has unsatisfied stability performance, not good as others, sensitive to environmental conditions i.e. gets outstanding performance with good conditions, but reduces yield a lot when meets adverse conditions.

As for HSC, it is simpler compared to a_i and CV, lower HSC value means better yield and stability performance.

Results and Discussion

2016-Curcumin Concentration

The curcumin concentration of finger rhizome ranged from 0.65% to 5.05%, curcumin in mother rhizomes ranged from 0.74% to 5.48%, and the weighted average of mothers and fingers ranged from 0.70% to 5.27% (Table 20 and Figure 19). Difference in curcumin concentration were significant at the 0.001 level. Based on the Tukey test result, CL2 and CL7 had significantly lower curcumin concentrations than the top 6 varieties. CL9 had the highest curcumin concentration, which was greater than the concentrations in the other varieties. For mother rhizome, both CL5 and CL9 were significantly higher than other varieties. Environmental conditions of different blocks did not affect these characters significantly and replications had no significant difference.

2016-Curcumin Yield

The curcumin yield of finger rhizome ranged from 13.1 kg·ha⁻¹ to 79.4 kg·ha⁻¹, mother rhizome ranged from 22.8 kg·ha⁻¹ to 116.5 kg·ha⁻¹, and total yield ranged from 35.9 kg·ha⁻¹ to 195.9 kg·ha⁻¹ (Table 21 and Figure 19). The eight varieties had highly significant differences for curcumin yields at significance level of 0.005 or better. Based on the Tukey test result, CL7 had significantly lower curcumin yield compared to the top 5 varieties, and CL2 was significantly lower than the top 4 varieties. CL9 had the highest curcumin yield, which was different from the last 3 varieties, CL6, CL2 and CL7. CL2 and CL7 were removed from the field trial in 2017 due to low curcumin yield.

The block effect did not test significantly, which means that environmental

conditions of different blocks did not affect these characters significantly.

2017-Curcumin Concentration

The curcumin concentration of finger rhizomes ranged from 2.46% to 3.06%, mother rhizome ranged from 2.75% to 4.58%, and average concentration ranged from 2.81% to 3.82% (Table 22 and Figure 20). The seven varieties tested in 2017 had no significant differences for finger rhizomes. Significant difference appeared on mother rhizome curcumin concentration at significance level 0.05. CL9 had significantly higher curcumin concentration in mother rhizomes than the other rhizomes except CL8 at significance level 0.05 level. CL9 was also higher than other varieties as the average curcumin concentration at significance level 0.1 level. Environmental conditions of different blocks affected these characters significantly. But if planting location will affect curcumin concentration may need further investigation.

2017-Curcumin Yield

The curcumin yield of finger rhizome ranged from 26.6 kg·ha⁻¹ to 56.4 kg·ha⁻¹, mother rhizome is ranges from 25.4 kg·ha⁻¹ to 38.2 kg·ha⁻¹, and total yield is ranges from 55.0 kg·ha⁻¹ to 94.6 kg·ha⁻¹ (Table 23 and Figure 20). Total curcumin yields of the seven varieties did not test significantly different at significance levels 0.05 and 0.1. As for finger rhizome and mother rhizome, curcumin yields had difference at significance level 0.1. CA1, CL3 and CL10 had higher curcumin yield than the average curcumin yield and were retained for next field trial in 2018. CL9 just had acceptable curcumin yield, but in

the previous year, CL9 had the highest curcumin yield. For that reason, CL9 also was retained together with other three varieties.

2018-Curcumin Concentration

The curcumin concentration of finger rhizomes in the 2018 trial ranged from 3.34 % to 4.67 %, mother rhizome ranged from 4.56 % to 6.35 %, and the weighted average curcumin concentration for finger and mother rhizomes ranged from 3.99% to 5.50 % (Table 24 and Figure 21). Significant differences among the four varieties were recorded for finger, mother and average curcumin concentration at significance level 0.1.

Differences were significant at the level 0.05 for finger and the weighted average of total rhizome curcumin concentration. CL9 had the highest curcumin concentration, which was significantly higher than for the other three varieties. Environmental conditions of different blocks did not affect these characters significantly.

2018-Curcumin Yield

The curcumin yield of finger rhizome ranged from 90.7 kg·ha⁻¹ to 148.5 kg·ha⁻¹, mother rhizome ranged from 82.7 kg·ha⁻¹ to 145.9 kg·ha⁻¹, and total yield ranged from 181.8 kg·ha⁻¹ to 294.4 kg·ha⁻¹ (Table 25 and Figure 21). Finger rhizome did not differ significantly, mother rhizome curcumin yield was significant at the 0.05 level. And p value of total curcumin yields was very close to 0.05 (0.052), so they may have a strong tendency towards statistical significance. The total curcumin yield of CL9 was significantly different from CL3 at 0.1 significant level. Environmental conditions of

different blocks did not affect these characters significantly. CL9 had the highest curcumin yield.

Curcumin I, II & III

Diferuloylmethane (DM), desmethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), also called curcumin I, curcumin II and curcumin III, respectively, were analyzed separately. In this thesis, finger rhizome and mother rhizome were combined to show the total curcumin yield and curcumin concentration. Significant differences among varieties were observed for DM yields (Table 28). Table 28 gives the DM, DMC and BDMC yields ($\text{kg}\cdot\text{ha}^{-1}$) in each of the nine turmeric varieties tested during the three years, Yield data of CA1, CL2, CL3, CL5, CL6, CL7, CL8, CL9 were from curcuminoids analysis in 2016. CL10 curcuminoids data was obtained 2017. CL9 had the highest DM yield and it was significant different from CL8, CL6, CL10, CL2 and CL7. CL7 had the lowest DM yield. There were significant differences among varieties for DMC yields. CL9 had the highest DMC yield, and it had significantly higher DMC yield than CL8, CL6, CL10, CL2 and CL7. CL7 had the lowest DMC yield. There were also significant differences among varieties for BDMC. CL9 had the highest BDMC yield, and it was significantly higher than the BDMC yield of CL6, CL10, CL2 and CL7. CL7 had the lowest BDMC yield.

There was no significant differences among varieties for DMC yield as a percentage of total curcuminoids, but there were significant differences for DM and BDMC (Table

27). The highest DM percentage were recorded for CL2 and CL7; The highest BDMC as a proportion of total curcuminoids was recorded for CL8, followed by CL5 and CA1. They were significantly higher than others. Overall, these nine varieties within this project, DMC proportions of total rhizome were extremely same, close to 18.6 %, and p value was 0.915 (close to 1). On the other side, DM and BDMC concentrations were highly variable for different varieties. CL2 and CL7 had the highest DM proportions (71.5 % and 69.0 %), while they got the lowest BDMC proportions (10.8 % and 11.4 %). The content of DM, DMC and BDMC varied from different varieties, and this may be true for other turmeric varieties.

Covariate Test of Curcumin Yield

To reduce the disease effects on some concentrated plots, covariate analysis was carried out to see if the disease likely altered the results in terms of curcumin yield. The result of the covariate analysis was compared with the original test result (Table 25). The covariate analysis reduced the differences between varieties. However, the rank in yield from high to low did not change based on covariate test, it only narrowed the gaps between the high yield and low yield. In other words, covariate test gave smaller difference of varieties compared to unadjusted result. CL9 has the highest curcumin yield, but it was not significantly higher than other varieties.

To explain this slight change, the four heavily diseased plots, 13, 14, 24 and 34, all only belonged to two varieties, CL3 and CL10, which were the varieties than had the

lowest average yield. Without the effect of disease, CL3 and CL10 would have performed better, and thus there would have been smaller differences between the varieties.

Curcumin Yield Stability Analysis

Figure 22 showed curcumin yields of varieties over three years had huge difference. Based upon comparison of CV%, CL3 had the best stability for curcumin yield, followed by CA1, CL9 and CL10 (Figure 23). But the yield of CL3 was the lowest. CL9 had the highest yield. Figure 24 shows CL3 had the best stability based upon ai method, followed by CA1, CL10 and CL9. CA1 had the best stability based upon the HSC method, followed by CL3, CL10 and CL9 (Table 26).

Comparing the three stability test results, CL9 had the worst stability, compared to the other three varieties. CL3 had the best stability followed by CA1 and CL10. Besides, CL3 and CA1 could responded to environments very well, and are suited for more different conditions. CL9 and CL10 did not have as good stability as the other two varieties, suggesting that they are very sensitive to environments. They will get outstanding performance under good conditions, but yield will be reduced a lot when they meet adverse conditions.

Conclusion

CL3 had the best stability, but its yield and curcumin performances were not outstanding. Hence, CL3 likely is not a potential valuable variety for further research.

The rankings for the yield of curcumin I, II and III, are similar to the rankings of the

pooled curcuminoid yields of varieties. Therefore, high curcumin yield means good yields on all three curcuminoids. DM and BDMC concentrations were highly vary from different varieties. CL2 and CL7 had the highest DM proportions (71.5 % and 69.0 %), while they had the lowest BDMC proportions (10.8 % and 11.4 %). The proportions of DM, DMC and BDMC vary from different varieties, and this may be true for other turmeric varieties.

CL2 and CL7 had extremely low curcumin concentration and low curcumin yields. CL9 had the highest curcumin concentration and highest curcumin yield in 2016 and 2018, followed by CA1. Other varieties are all close or lower than average performance.

Overall, if curcumin is the primary target, CL9 should be the best variety. But compared to CA1, it showed bigger fluctuation during three years. CL9 appears to be sensitive to environmental conditions, but it could produce better curcumin yield in conducive environments. Especially in 2017, CL9 was distributed mostly on the east side, which had worse disease compared to west side. This likely was an important part of reason why CL9 got obvious bad performance in 2017. In other words, CL9 has huge potential on curcumin yield. And it could be a primary variety source. CA1 has good curcumin yield and acceptable stability, also could be an acceptable variety. Further field trials could be continued to try assessing the yield stability and yield potential of these varieties.

Table 1. The yield percentage of turmeric rhizome in global area.

Countries	Percentage of Global Market
	%
India	78
China	8
Myanmar	4
Nigeria	3
Bangladesh	3

Note: Data got from Agricultural Situation in India and Directorate General of Commercial Intelligence and Statistics (Angles et al., 2011)

Table 2. The production and output of turmeric in India.

Year	Total Production	Export Quantity
	10 ³ Mg	10 ³ Mg
1950-1951†	120	4.8
1961-1961	155	6.2
1970-1971	203	8.1
1980-1981	280	11.2
1990-1991	490	19.6
2000-2001	654	26.2
2009-2010	888	55.9
2012-2013‡	971	---
2013-2014	1229	---
2014-2015	852	---
2015-2016	943	---
2016-2017	1132	---

†Source of data from 1950 to 2010: (Parthasarthy et al., 2007)

‡Source of data from 2012 to 2017: (Kumar and Sankaran, 1998)

Note: Data got from Agricultural Situation in India and Directorate General of Commercial Intelligence and Statistics.

Table 3. 10 main export destinations of turmeric from India during 2008 and 2010.

Countries	Quantity	
	2008-2009	2009-2010
	10 ³ Mg	10 ³ Mg
United Arab Emirate	5.8	6.1
Bangladesh	4.6	4.9
Iran	5.1	4.6
Malaysia	4.1	4.3
USA	4.4	4.0
Japan	3.5	3.1
Sri Lanka	3.2	3.0
UK	3.1	2.9
South Africa	2.1	1.7
Egypt	2.1	1.7
World	55.9	56.4

Note: Data got from Directorate General of Commercial Intelligence and Statistics

(Kumar and Sankaran, 2012).

Table 4. Regular experimental conditions for the HPLC separation (Snyder et al., 2012).

Separation Variable	Preferred Initial Choice
Column	
Dimensions (length, ID)	15 * 0.46 cm
Particle size	5 μ m ^a
Stationary phase	C ₈ or C ₁₈
Mobile phase	
Solvents A and B	Buffer-acetonitrile
%B	50-100% ^b
Flow rate	1.5-2.0 mL·min ⁻¹
Temperature	35-45°C
Sample size	
Volume	<25 μ L
Weight	<100 μ g

Table 5. The origin of 9 turmeric varieties in this project.

Accession	Origin	Accession	Origin
CA1	Unknown	CL8	South Asia
CL2	Unknown	CL9	Vietnam
CL3	Hawaii	CL10	Unknown
CL5	India		
CL6	India		
CL7	Korea		

Table 6. Soil test results during 2016 and 2018. Auburn Agronomy Farm.

Year	pH	Nutrition			
		Phosphorus	Potassium	Magnesium	Calcium
		Meq·100g soil ⁻¹	Meq·100g soil ⁻¹	Meq·100g soil ⁻¹	Meq·100g soil ⁻¹
2016	5.7	0.09	0.07	0.61	1.83
2017	5.8	0.20	0.08	0.55	1.55
2018	6.1	0.03	0.04	0.87	1.60

Table 7. Shapiro-Wilk test and Bartlett test results for fresh rhizome yield, rhizome dry weight yield and percent dry weight yield in 2016. Units are $\text{kg}\cdot\text{ha}^{-1}$, $\text{kg}\cdot\text{ha}^{-1}$ and %.

Year	Performance	P value	
		Shapiro-Wilk test	Bartlett test
2016	Fresh Rhizome Yield	0.751	0.760
	Rhizome Dry Weight Yield	0.763	0.872
	Percent Dry Weight of Rhizome	0.369	0.667
2017	Fresh Rhizome Yield	0.180	0.779
	Rhizome Dry Weight Yield	0.269	0.535
	Percent Dry Weight of Rhizome	0.349	0.265
2018	Fresh Rhizome Yield	0.674	0.552
	Rhizome Dry Weight Yield	0.071	0.284
	Percent Dry Weight of Rhizome	0.462	0.234

Note: All these items are for total yield performance; all rhizome yield data were adjusted to 10 % moisture content base on industry standard.

Table 8. Turmeric rhizome fresh yield in 2016. Auburn Agronomy field, AL.

Variety	Fresh Finger Rhizome Yield	Fresh Mother Rhizome Yield	Total Fresh Rhizome Yield
	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹
CA1	10750	10730	21480
CL2	17390	17330	34710
CL3	11230	11260	22440
CL5	11620	11800	23450
CL6	7550	7510	15050
CL7	12890	12540	25490
CL8	8280	8330	16610
CL9	11280	11200	22110
Mean	11370	11340	22670
S	2810	2280	5590
p Variety	0.110	0.080	0.377
p Blocks	0.565	0.212	0.343
CV %	24.7	24.5	24.7

Table 9. Turmeric rhizome dry weight yield in 2016. Auburn Agronomy field, AL.

Variety	Dry Finger Rhizome Yield†	Dry Mother Rhizome Yield	Total Dry Rhizome Yield
	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹
CA1	1750 bc	2220 abc	3970 bc
CL2	3100 a	3500 bc	6600 a
CL3	1810 bc	2320 ab	4130 bc
CL5	1340 c	2040 abc	3380 bc
CL6	880 c	1250 abc	2120 c
CL7	2500 ab	2600 c	5100 ab
CL8	1430 bc	1740 abc	3170 bc
CL9	1760 bc	2310 a	4070 b
Mean	1820	2250	4070
S	690	650	1340
p Variety	0.002	0.028	0.002
p Blocks	0.584	0.124	0.156
CV %	38.1	29.1	32.9

†0.05 significant level, same letters or no letter do not differ at the 0.05 level of probability as determined by the Tukey HSD test.

Note: Rhizome dry yields were adjusted to 10 % moisture content based on industry standard.

Table 10. Percent dry weight of finger, mother and total rhizome yields in 2016. Auburn agronomy field.

Variety	Finger Rhizome Percent Dry Weight†	Mother Rhizome Percent Dry Weight	Average Total Rhizome Percent Dry Weight†
	%	%	%
CA1	16.3 bc	20.7 a	18.5 bc
CL2	17.8 ab	20.2 ab	19.0 a
CL3	16.1 bc	20.6 a	18.4 bc
CL5	11.5 d	17.3 ab	14.4 bc
CL6	11.6 d	16.6 b	14.1 c
CL7	19.4 a	20.7 a	20.0 ab
CL8	17.3 bc	20.9 a	19.1 bc
CL9	15.6 c	20.6 a	18.4 b
Mean	15.7	19.7	17.7
S	2.8	1.7	2.2
p Variety	0.001	0.031	0.001
p Blocks	0.207	0.830	0.207
CV %	18.0	8.7	12.5

†0.05 significant level, same letters or no letter do not differ at the 0.05 level of probability as determined by the Tukey test.

Table 11. Turmeric fresh rhizome yield in 2017. Auburn agronomy field, AL.

Variety	Fresh Finger Rhizome Yield	Fresh Mother Rhizome Yield	Total Fresh Rhizome Yield
	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹
CA1	10520	5190	15750
CL3	8020	5000	13040
CL5	10340	4580	14900
CL6	7310	4180	11470
CL8	7020	3120	10150
CL9	7150	3120	10290
CL10	7830	4580	12440
Mean	8310	4250	12580
S	1380	780	2000
p Variety	0.055	0.189	0.131
p Blocks	0.835	0.051	0.592
CV%	16.6	18.3	15.9

Table 12. Turmeric rhizome dry weight yield in 2017. Auburn Agronomy field, AL.

Variety	Dry Finger Rhizome Yield†	Dry Mother Rhizome Yield	Total Dry Rhizome Yield†
	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹
CA1	2030 a	1110	3140 a
CL3	1520 abc	1050	2570 abc
CL5	1240 bc	760	2000 bc
CL6	940 c	730	1670 c
CL8	1440 abc	730	2170 abc
CL9	1210 bc	640	1850 bc
CL10	1640 ab	1130	2770 ab
Mean	1430	880	2310
S	320	190	490
p Variety	0.016	0.136	0.029
p Blocks	0.758	0.106	0.713
CV%	22.7	22.0	21.4

†0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey HSD test.

Note: Rhizome dry yields were adjusted to 10 % moisture content based on industry standard.

Table 13. Percent dry weight of finger, mother and total rhizome yields in 2017. Auburn agronomy field.

Variety	Finger Rhizome Percent Dry Weight†	Mother Rhizome Percent Dry Weight	Weighted Average Total Rhizome Percent Dry Weight†
	%	%	%
CA1	19.3 a	21.3 bc	19.9 bc
CL3	19.0 a	20.9 bc	19.7 bc
CL5	12.0 c	16.5 d	13.4 d
CL6	12.9 c	17.5 d	14.6 d
CL8	20.5 a	23.5 ab	21.4 ab
CL9	16.9 b	20.6 c	18.0 c
CL10	21.0 a	24.7 a	22.3 a
Mean	17.4	20.7	18.5
S	3.6	2.9	3.4
p Variety	0.001	0.001	0.001
p Blocks	0.099	0.493	0.099
CV%	20.8	14.2	18.2

†0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey HSD test.

Table 14. Turmeric rhizome fresh yield in 2018. Auburn Agronomy field, AL.

Variety	Fresh Finger Rhizome Yield†	Fresh Mother Rhizome Yield	Total Fresh Rhizome Yield	Total Rhizome Yield Adjusted for Covariate
	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹
CA1	25710 a	9680 b	35110 a	31220
CL3	12580 b	6360 b	18810 b	23820
CL9	22500 ab	14040 a	36350 a	32940
CL10	13290 b	10240 ab	23340 ab	23910
Mean	18520	10080	28400	27970
S	6590	3150	8670	4800
p Variety	0.023	0.014	0.014	0.091
p Blocks	0.175	0.358	0.227	0.421
CV%	35.6	31.2	30.5	17.1

†0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey HSD test.

Table 15. Turmeric rhizome dry weight yield in 2018. Auburn Agronomy field, AL.

Variety	Dry Finger Rhizome Yield†	Dry Mother Rhizome Yield	Total Dry Rhizome Yield†	Total Rhizome Yield Adjusted for Covariate
	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹
CA1	4140	1830 ab	5970	5550
CL3	2230	1370 b	3590	4330
CL9	3150	2300 a	5450	5070
CL10	2570	2310 a	4880	4950
Mean	3020	1950	4970	4980
S	836	448	1024	502
p Variety	0.051	0.012	0.066	0.301
p Blocks	0.173	0.148	0.219	0.289
CV%	27.7	23.0	20.6	10.1

†0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey HSD test.

Note: Rhizome dry yields were adjusted to 10 % moisture content based on industry standard.

Table 16. Percent dry weight of finger, mother and total rhizome yields in 2018. Auburn agronomy field.

Variety	Finger Rhizome Percent Dry Weight†	Mother Rhizome Percent Dry Weight	Weighted Average Total Rhizome Percent Dry Weight†
	%	%	%
CA1	16.1 bc	18.9 ab	17.0 bc
CL3	17.7 ab	21.5 a	19.1 ab
CL9	14.0 c	16.4 b	15.0 c
CL10	19.3 a	22.6 a	20.9 a
Mean	16.8	19.9	18.0
S	2.3	2.8	2.6
p Variety	0.011	0.028	0.018
p Blocks	0.551	0.492	0.531
CV%	13.5	14.0	14.2

†0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey test.

Table 17. Turmeric total fresh rhizome yields in 2016, 2017 and 2018. Auburn agronomy field, AL. 4 varieties.

Year	Fresh Rhizome Yield				Mean
	CA1	CL3	CL9	CL10	
	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹
2016	21480	22440	22110	NA	22010
2017	15750	13040	10290	12440	12880
2018†	31220	23820	32940	23910	27970
Mean	22820	19770	21780	18180	20950
S	9945	4740	13048	7707	7801
CV %	41.2	26.2	56.9	43.1	37.0
ai	1.27	0.61	1.67	0.99	1.14
HSC %	39.0	42.4	57.5	56.1	47.9

†data were adjusted by covariate test.

Table 18. Turmeric total rhizome dry yields of four varieties in 2016, 2017 and 2018.

Auburn agronomy field, Alabama.

Year	Dry Rhizome Yield				Mean
	CA1	CL3	CL9	CL10	
	kg ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹
2016	3970	4130	3190	NA	3760
2017	3140	2570	1850	2770	2580
2018†	5550	4330	5070	4950	4980
Mean	4220	3680	3370	3860	3770
S	1460	790	1820	1490	1200
CV %	33.4	23.1	52.0	38.9	31.7
ai	1.01	0.75	1.48	1.12	1.09
HSC %	48.3	51.6	51.5	67.7	54.8

†data were adjusted by covariate test.

Note: All rhizome dry yield data has adjusted to 10 % moisture content based on industry standard.

Table 19. Shapiro-Wilk test and Bartlett test results for curcumin concentration and curcumin yield in 2016. Units are % and kg·ha⁻¹.

Year	Performance	P value	
		Shapiro-Wilk test	Bartlett test
2016	Curcumin Concentration	0.083	0.474
	Curcumin Yield	0.065	0.054
2017	Curcumin Concentration	0.343	0.242
	Curcumin Yield	0.858	0.298
2018	Curcumin Concentration	0.572	0.756
	Curcumin Yield	0.613	0.279

Note: All these items are for total yield performance; all rhizome yield data were adjusted to 10 % moisture content base on industry standard; curcumin concentration and yield are for total rhizome.

Table 20. Finger, mother rhizome and average curcumin concentrations in turmeric rhizomes in 2016. Auburn Agronomy field, AL.

Variety	Finger Curcumin Concentration†	Mother Curcumin Concentration	Total Rhizome Weighted Average Curcumin Concentration
	%	%	%
CA1	3.31 b	3.71 bc	3.51 c
CL2	0.65 c	0.74 d	0.70 d
CL3	3.49 b	3.44 c	3.47 c
CL5	3.58 b	5.28 a	4.43 b
CL6	3.59 b	4.29 b	3.94 c
CL7	0.57 c	0.87 d	0.72 d
CL8	3.00 b	3.67 bc	3.34 c
CL9	5.05 a	5.48 a	5.27 a
Mean	2.91	3.44	3.17
S	1.54	1.78	1.64
p Variety	0.001	0.001	0.001
p Blocks	0.708	0.287	0.602
CV%	53.0	51.9	51.8

†0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey HSD test.

Table 21. Finger, mother rhizome and total curcumin yields in turmeric rhizomes in 2016.

Auburn Agronomy field, AL.

Variety	Finger Curcumin Yield[†]	Mother Curcumin Yield	Total Curcumin Yield
	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹
CA1	53.3 ab	72.4 abc	125.7 ab
CL2	18.0 cd	23.4 c	41.4 cd
CL3	56.9 ab	72.7 abc	129.6 ab
CL5	33.5 bcd	79.6 ab	113.1 ab
CL6	30.6 bcd	49.0 bc	79.6 bcd
CL7	13.1 d	22.8 c	35.9 d
CL8	39.7 abc	61.9 abc	101.6 abc
CL9	79.4 a	116.5 a	195.9 a
Mean	40.6	62.3	102.9
S	21.9	30.9	51.8
p Variety	0.002	0.003	0.004
p Blocks	0.455	0.181	0.195
CV%	53.9	49.7	50.4

[†]0.05 significant level, same letters do not differ at the 0.05 level of probability as

determined by the Tukey HSD test.

Note: all rhizome yield data were adjusted to 10 % moisture content base on industry standard.

Table 22. Curcumin concentration of turmeric rhizomes in 2017. Auburn Agronomy field, AL.

Variety	Finger Curcumin Concentration†	Mother Curcumin Concentration	Total Rhizome Weighted Average Curcumin Concentration
	%	%	%
CA1	2.74	3.47 bc	3.10
CL3	2.46	3.18 bc	2.82
CL5	2.83	3.35 bc	3.09
CL6	2.75	3.63 b	3.19
CL8	2.60	3.87 ab	3.24
CL9	3.06	4.58 a	3.82
CL10	2.86	2.75 c	2.81
Mean	2.76	3.55	3.15
S	0.19	0.58	0.34
p Variety	0.381	0.001	0.061
p Blocks	0.004	0.012	0.005
CV%	7.0	16.2	10.7

†0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey HSD test.

Table 23. Curcumin yields of turmeric rhizome in 2017. Auburn Agronomy field, AL.

Variety	Finger Curcumin Yield	Mother Curcumin Yield	Total Curcumin Yield
	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹
CA1	56.4	38.2	94.6
CL3	37.3	33.1	70.4
CL5	35.4	25.4	60.8
CL6	26.6	28.4	55.0
CL8	37.7	28.4	66.1
CL9	37.8	28.7	66.5
CL10	46.8	30.8	77.6
Mean	39.7	30.4	70.1
S	9.4	4.2	12.9
p Variety	0.086	0.695	0.179
p Blocks	0.265	0.013	0.119
CV%	23.7	13.7	18.4

Table 24. Curcumin concentration in turmeric rhizomes in 2018. Auburn Agronomy field,

AL.

Variety	Finger Curcumin Concentration†	Mother Curcumin Concentration	Average Curcumin Concentration
	%	%	%
CA1	3.37 b	5.59	4.31 b
CL3	3.65 b	4.69	4.12 b
CL9	4.67 a	6.35	5.50 a
CL10	3.34 b	4.56	3.99 b
Mean	3.76	5.30	4.48
S	0.62	0.84	0.69
p Variety	0.009	0.088	0.013
p Blocks	0.451	0.572	0.672
CV%	16.6	15.8	15.5

†0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey test.

Table 25. Curcumin yields in turmeric rhizomes in 2018. Auburn Agronomy field, AL.

Variety	Finger Curcumin Yield†	Mother Curcumin Yield	Total Curcumin Yield	Total Curcumin Yield adjusted for Covariate
	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹
CA1	138.8	102.2 ab	241.0	224.0
CL3	99.2	82.7 b	181.8	197.2
CL9	148.5	145.9 a	294.4	278.8
CL10	90.7	104.7 ab	195.4	198.3
Mean	119.3	108.9	228.2	224.6
S	28.6	26.6	50.9	38.2
P Variety	0.117	0.036	0.052	0.132
P Blocks	0.226	0.171	0.306	0.153
CV%	24.0	24.4	22.3	17.0

†0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey test.

Table 26. Curcumin yields in 2016, 2017 and 2018 in Auburn agronomy field, AL. 4

varieties.

Year	Curcumin Yield				Mean
	CA1	CL3	CL9	CL10	
	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹
2016	125.7	129.6	195.9	NA	150.4
2017	85.1	63.4	59.9	69.8	69.5
2018	240.0	181.8	294.4	195.4	228.2
2018(Cov)†	224.0	197.2	278.8	198.3	224.6
Mean	168.7	143.0	207.3	154.5	168.2
S	80.3	59.3	117.7	88.8	79.4
CV %	53.5	47.5	64.2	67.0	53.1
ai	1.22	0.66	1.52	1.24	1.16
HSC %	15.4	23.0	51.0	31.7	30.3

† data were adjusted by covariate test.

Note: Curcumin yield is total curcumin yield.

Table 27. Proportion of Diferuloylmethane (DM), Desmethoxycurcumin (DMC) and Bisdemethoxycurcumin (BDMC) as a proportion of total curcuminoids in 9 turmeric varieties included in trials.

Variety	DM	DMC	BDMC
	Proportion [†]	Proportion	Proportion
	%	%	%
CA1	59.4 bc	22.4	18.1 c
CL2	71.5 a	17.9	10.8 d
CL3	63.6 b	17.6	18.6 c
CL5	62.5 bc	17.3	20.3 abc
CL6	62.9 bc	18.0	19.1 bc
CL7	69.0 a	19.6	11.4 d
CL8	59.3 c	18.4	22.3 a
CL9	62.5 bc	18.7	18.8 c
CL10	63.3 bc	17.2	20.6 abc
Mean	63.8	18.6	17.8
S	4.0	1.6	4.0
p Variety	0.001	0.915	0.001
p Blocks	0.640	0.278	0.644
CV%	6.3	8.7	22.8

[†]0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey HSD test.

Note: Concentration data of varieties are from 2016 except for CL10 which is from.

Table 28. Diferuloylmethane (DM), Desmethoxycurcumin (DMC) and Bisdemethoxycurcumin (BDMC) curcuminoid yields in all nine turmeric varieties included in trials.

Variety	DM Yield†	DMC Yield	BDMC Yield
	kg·ha ⁻¹	kg·ha ⁻¹	kg·ha ⁻¹
CA1	64.4 abcd	24.3 ab	19.7 ab
CL2	29.6 cd	7.4 c	4.4 c
CL3	82.4 abc	22.8 ab	24.3 ab
CL5	88.7 ab	24.5 ab	28.8 ab
CL6	50.1 bcd	14.3 bc	15.2 bc
CL7	24.8 d	7.0 c	4.1 c
CL8	70.6 bcd	21.9 bc	26.5 ab
CL9	122.4 a	36.6 a	36.9 a
CL10	65.0 bcd	18.8 c	16.1 c
Mean	66.4	19.7	19.6
S	30.1	9.3	10.9
p Variety	0.015	0.009	0.002
p Blocks	0.197	0.137	0.196
CV%	45.3	47.0	55.9

†0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey test.

Note: Yield data of varieties is from 2016; CL10 got from 2017.

Table 29. Diseased rhizome weight and percent of diseased rhizome weight of 8 varieties

F test in 2016.

Variety	Diseased Rhizome Weight†	Percent of Diseased Rhizome Weight
	kg·ha ⁻¹	%
CA1	870	6.6 bc
CL2	3010	12.7 abc
CL3	620	3.8 c
CL5	2530	14.6 abc
CL6	2820	18.8 a
CL7	2460	16.3 ab
CL8	960	6.6 bc
CL9	1760	7.7 bc
Mean	1880	10.9
S	955	5.4
CV%	50.8	49.8

†0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey HSD test.

Table 30. Diseased rhizome weight and percent of diseased rhizome weight of 7 varieties

F test in 2017.

Variety	Diseased Rhizome Weight	Percent of Diseased Rhizome Weight
	kg·ha ⁻¹	%
CA1	268	1.9
CL3	405	3.4
CL5	472	3.4
CL6	411	4.3
CL8	292	3.3
CL9	395	4.4
CL10	265	2.4
Mean	358	3.3
S	82	0.9
p Variety	0.131	0.457
p Blocks	0.036	0.291
CV %	22.9	27.7

Table 31. Diseased rhizome weight and percent of diseased rhizome weight of 4 varieties

F test in 2018.

Variety	Diseased Rhizome Weight†	Percent of Diseased Rhizome Weight
	kg·ha ⁻¹	%
CA1	1370 c	4.2
CL3	4970 a	37.3
CL9	1750 c	5.2
CL10	3390 b	18.2
Mean	2870	16.2
S	1650	15.4
p Variety	0.014	0.522
p Blocks	0.001	0.609
CV %	57.6	95.3

†0.05 significant level, same letters do not differ at the 0.05 level of probability as determined by the Tukey test.

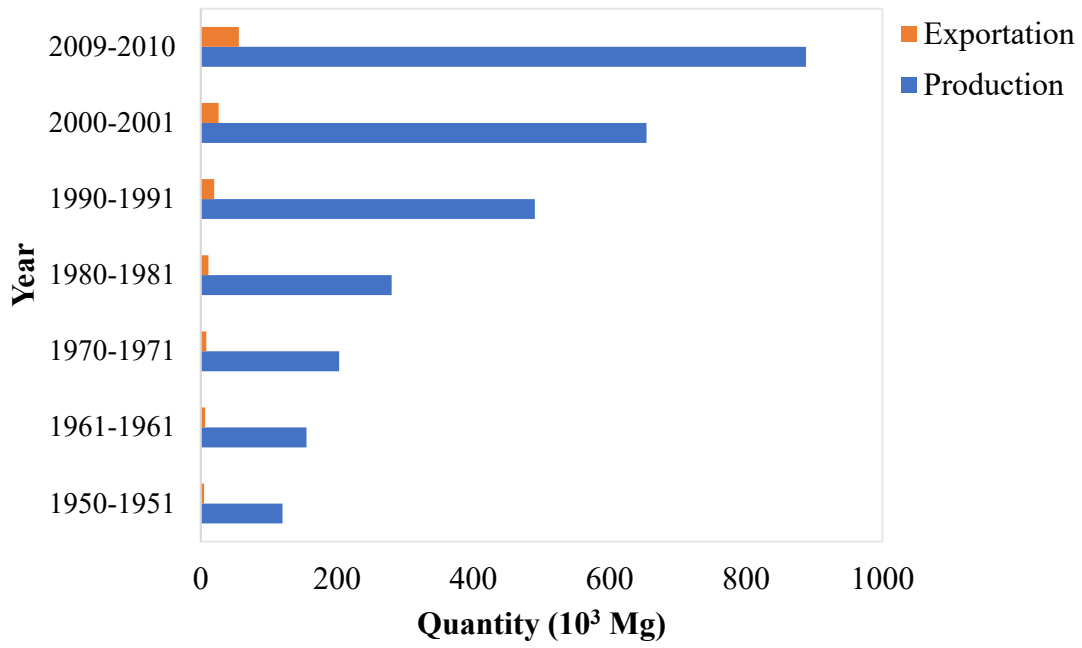


Figure 1. The production and exportation quantity of turmeric in India (Bansal et al., 2008).

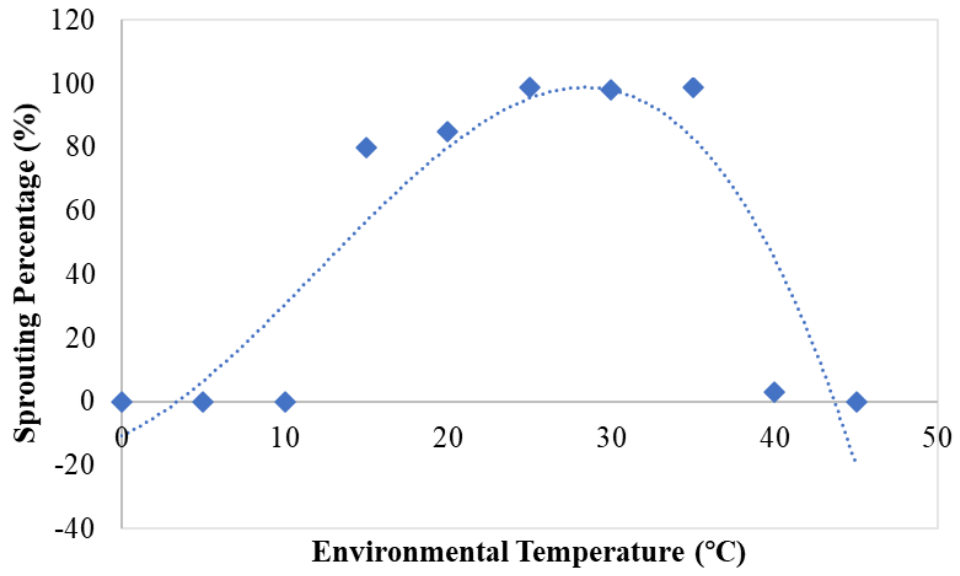


Figure 2. Turmeric seeding rhizome sprouting percentage based on environmental temperature (Ishimine et al., 2004).

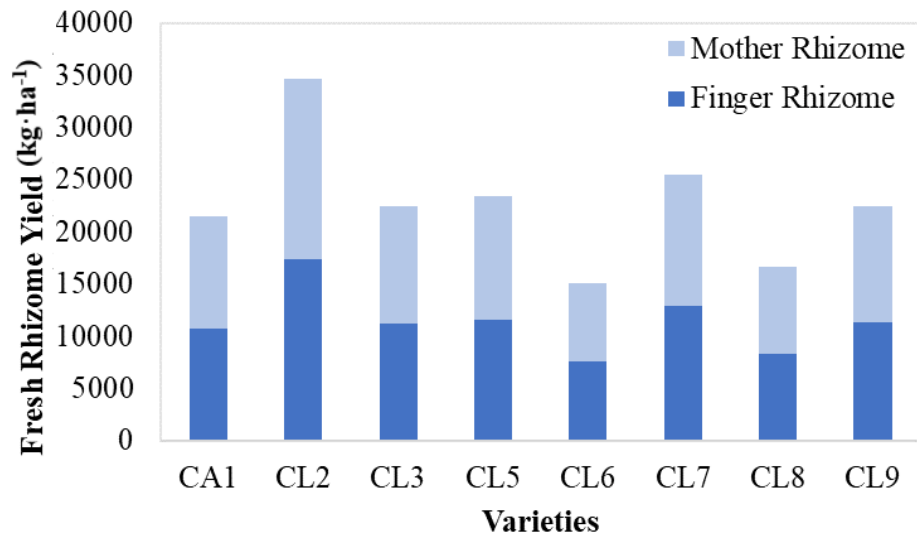


Figure 3. Fresh finger rhizome and mother rhizome yields in 2016. Auburn agronomy field.

Note: All rhizome yield data has adjusted to 10 % moisture content based on industry standard.

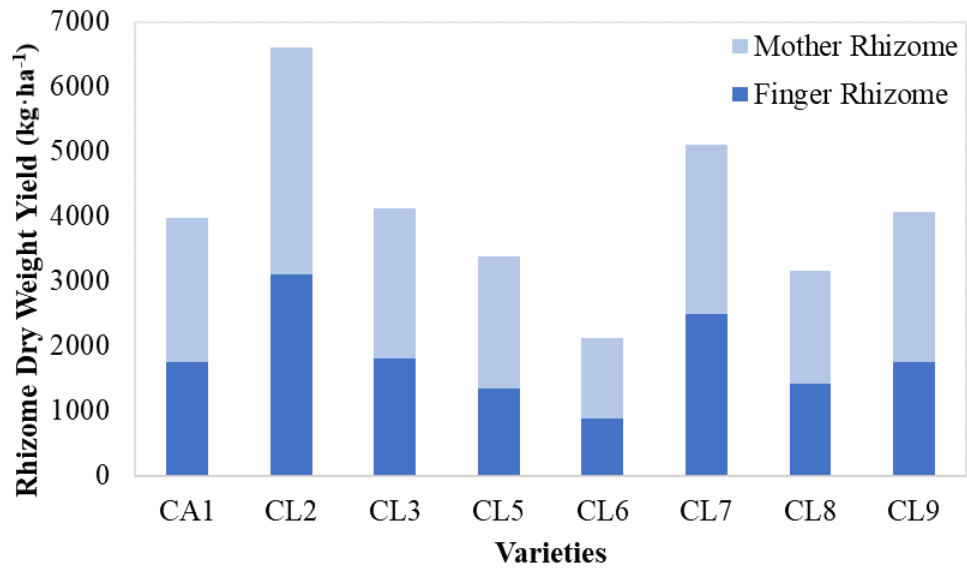


Figure 4: Turmeric finger and mother rhizomes dry weight yields in 2016. Auburn agronomy field.

Note: All rhizome yield data adjusted to 10 % moisture content based on industry standard.

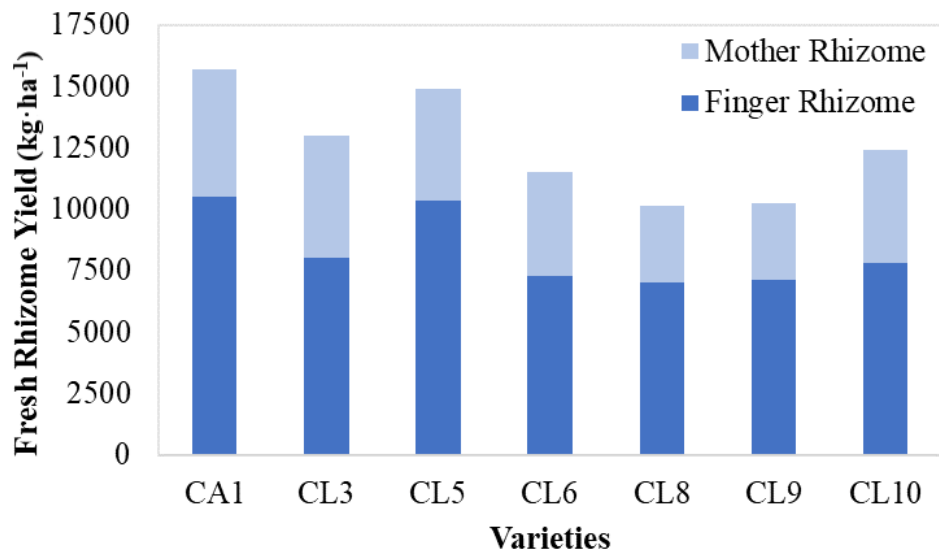


Figure 5. Fresh finger rhizome and mother rhizome yields in 2017. Auburn agronomy field.

Note: All rhizome yield data adjusted to 10 % moisture content based on industry standard.

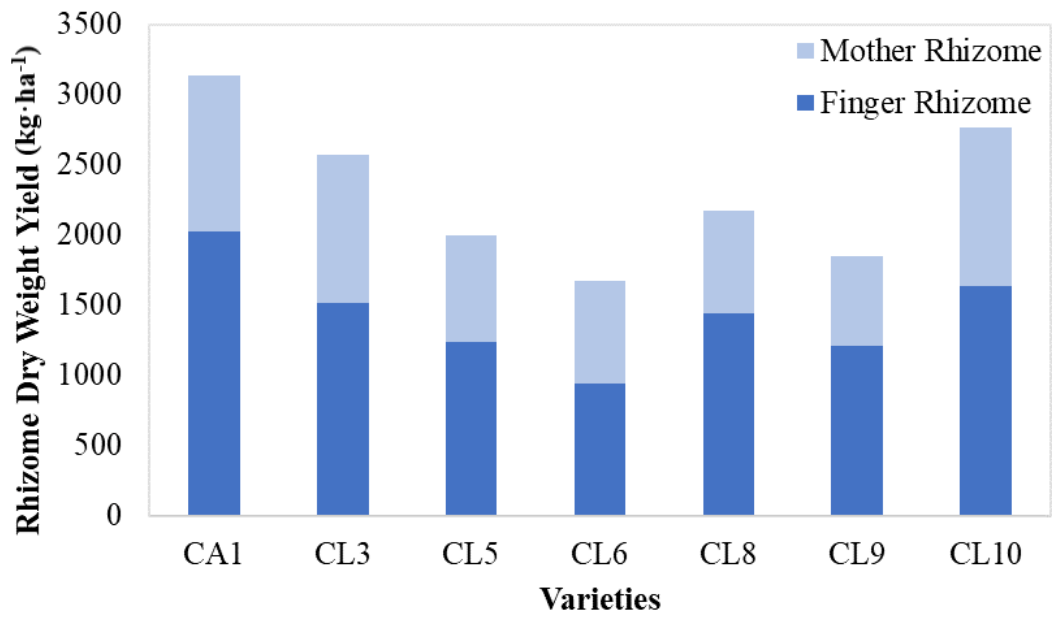


Figure 6. Turmeric finger and mother rhizomes dry weight yields in 2017. Auburn agronomy field.

Note: All rhizome yield data adjusted to 10 % moisture content based on industry standard.

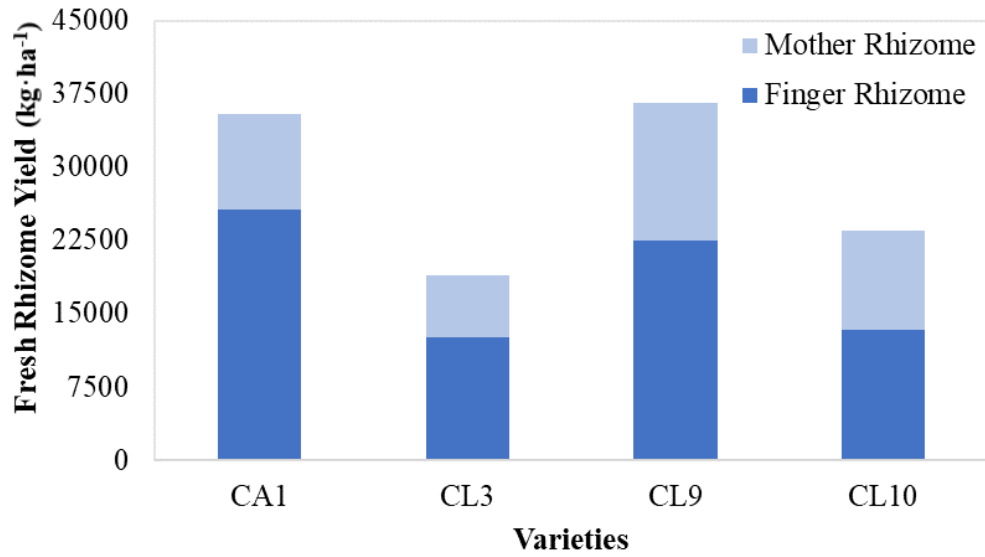


Figure 7. Fresh finger rhizome and mother rhizome yields in 2018. Auburn agronomy field.

Note: All rhizome yield data has adjusted to 10 % moisture content based on industry standard.

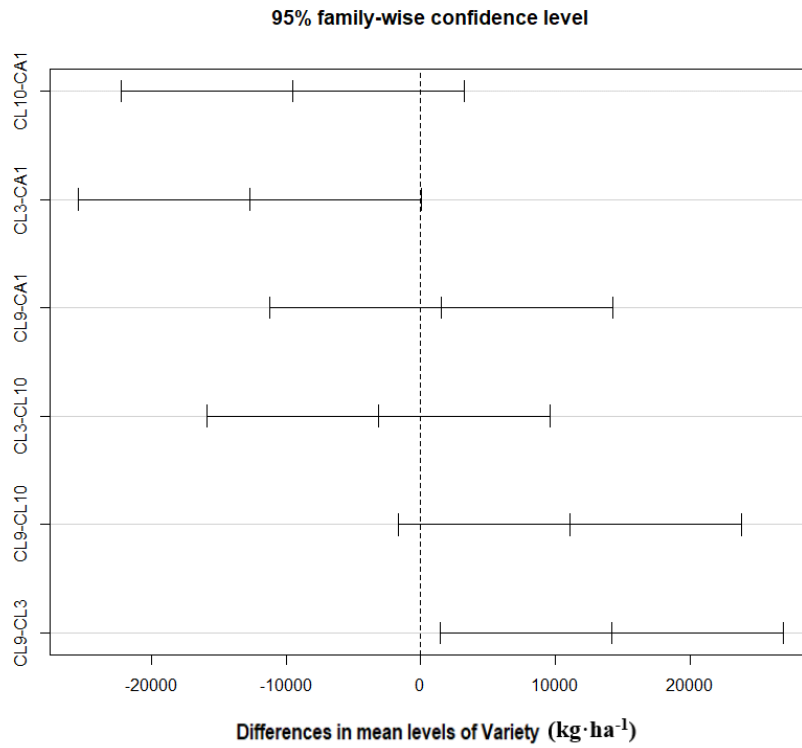


Figure 8. 95 % confidence intervals on differences between pairs of varieties for fresh weight yield in 2018.

Note: All rhizome yield data has adjusted to 10 % moisture content based on industry standard.

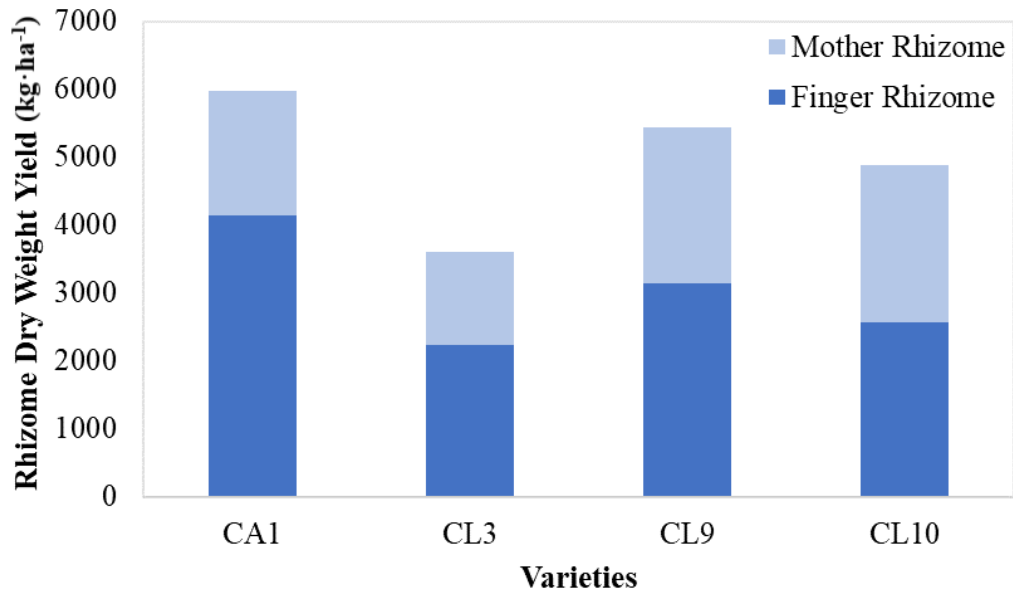


Figure 9. Turmeric finger and mother rhizomes dry weight yields in 2018. Auburn agronomy field.

Note: All rhizome yield data has adjusted to 10 % moisture content based on industry standard.

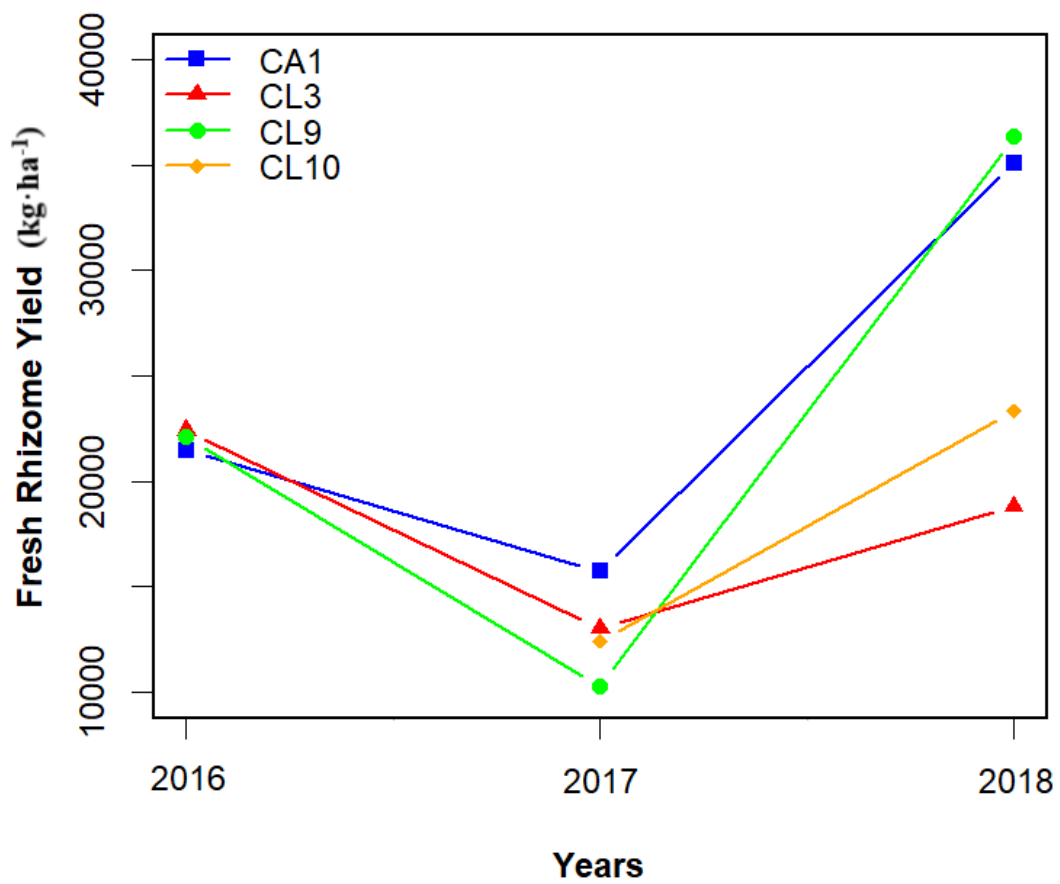


Figure 10. Fresh rhizome yield of four varieties in 2016, 2017 and 2018.

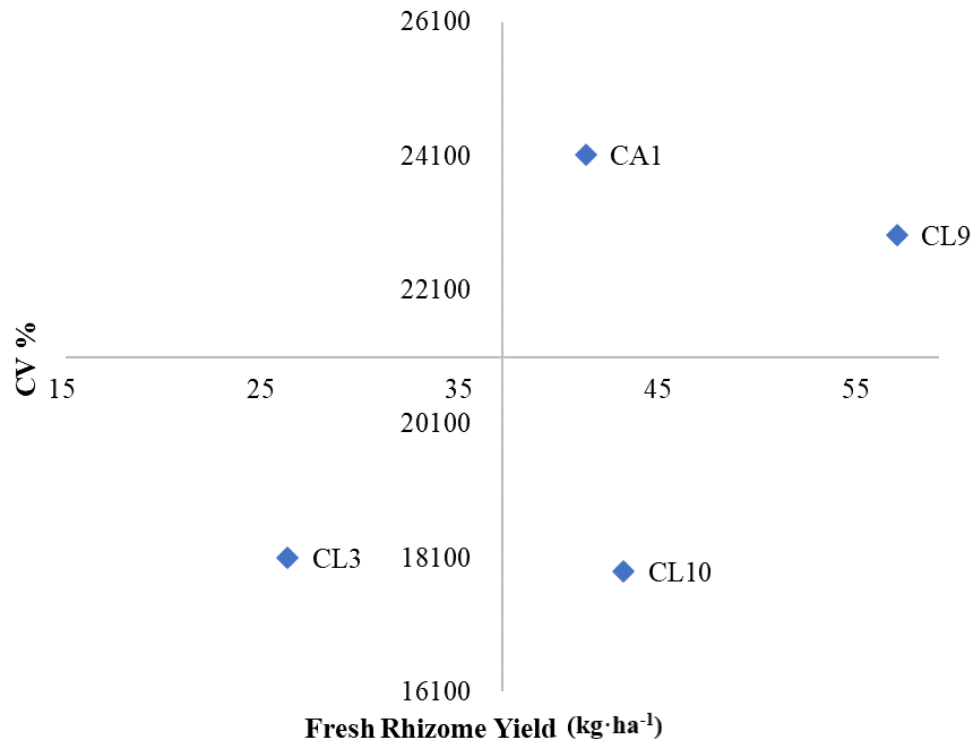


Figure 11. Four-quadrant diagram shows the CV % and fresh rhizome yield of four varieties, annual performance during 2016 and 2018.

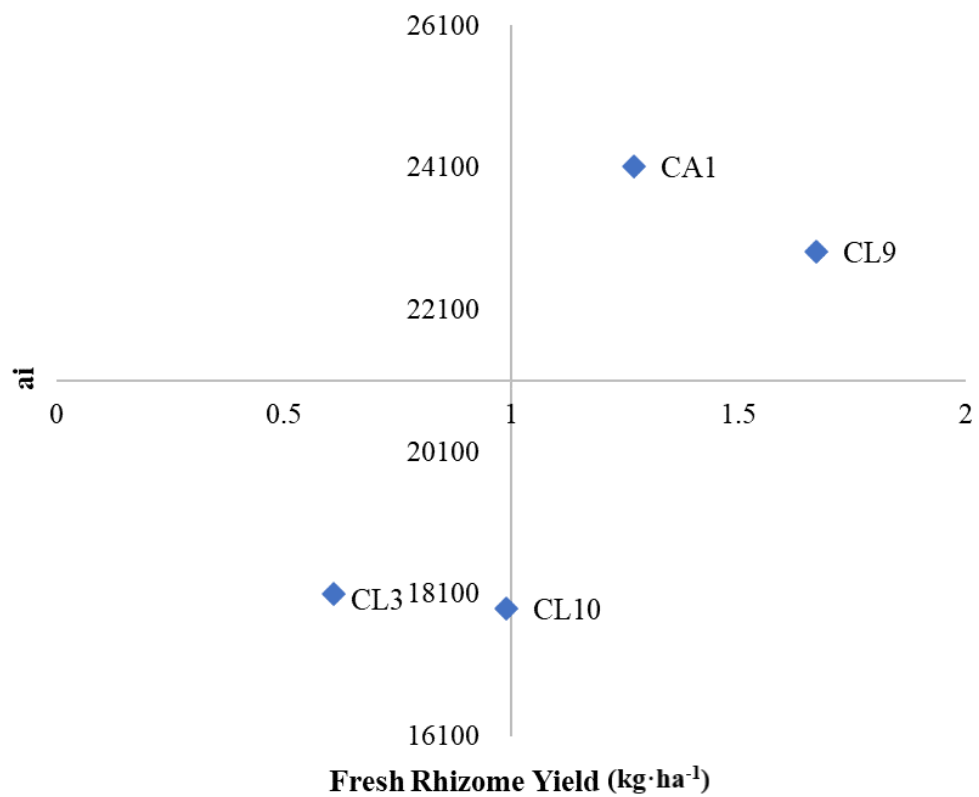


Figure 12. Four-quadrant diagram shows the ai value and fresh rhizome yield of four varieties, annual performance during 2016 and 2018.

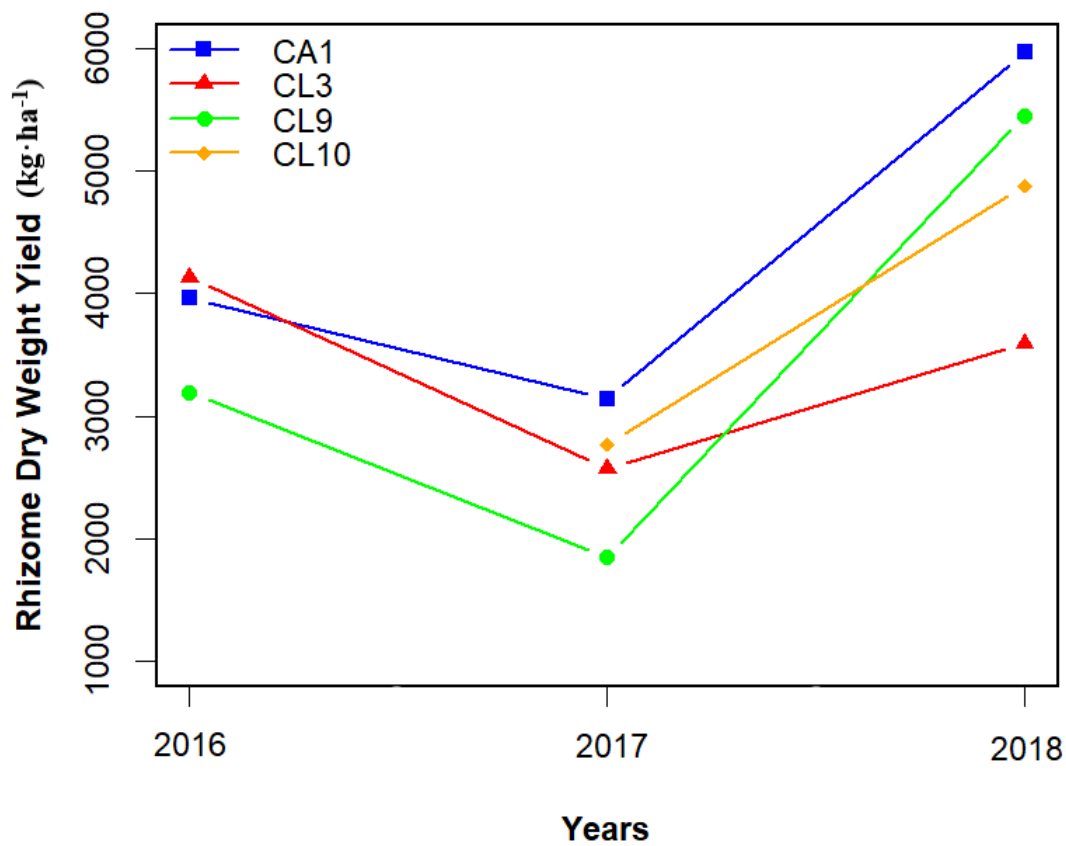


Figure 13. Total rhizome dry weight yield of 4 varieties in 2016, 2017 and 2018.

Note: All rhizome yield data has adjusted to 10 % moisture content based on industry standard.

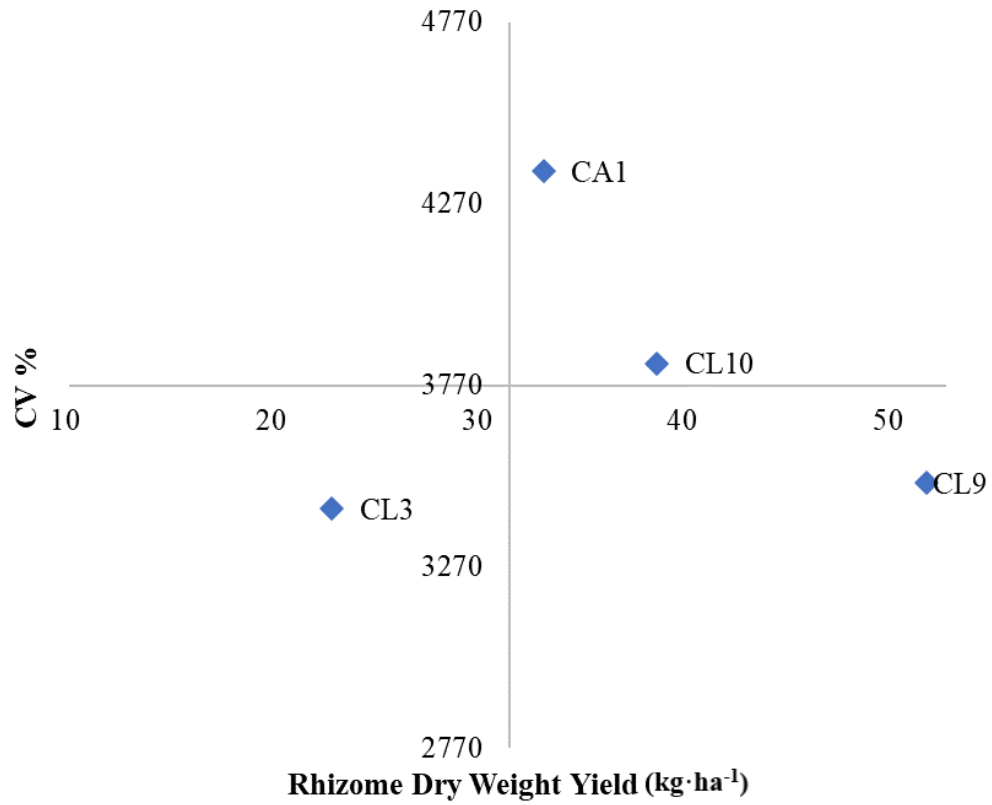


Figure 14. Four-quadrant diagram shows the CV % and rhizome dry weight yield of four varieties, annual performance during 2016 and 2018.

Note: All rhizome yield data has adjusted to 10 % moisture content based on industry standard.

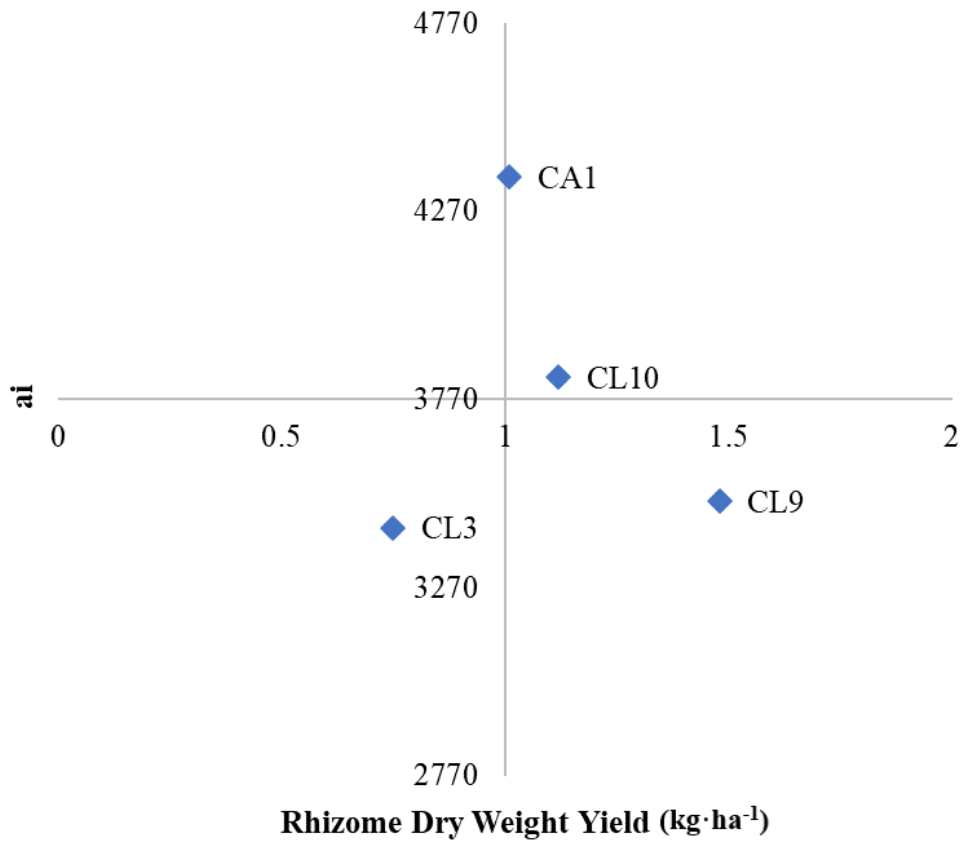


Figure 15. Four-quadrant diagram shows the ai value and rhizome dry weight yield of four varieties, annual performance during 2016 and 2018.

Note: All rhizome yield data has adjusted to 10 % moisture content based on industry standard.

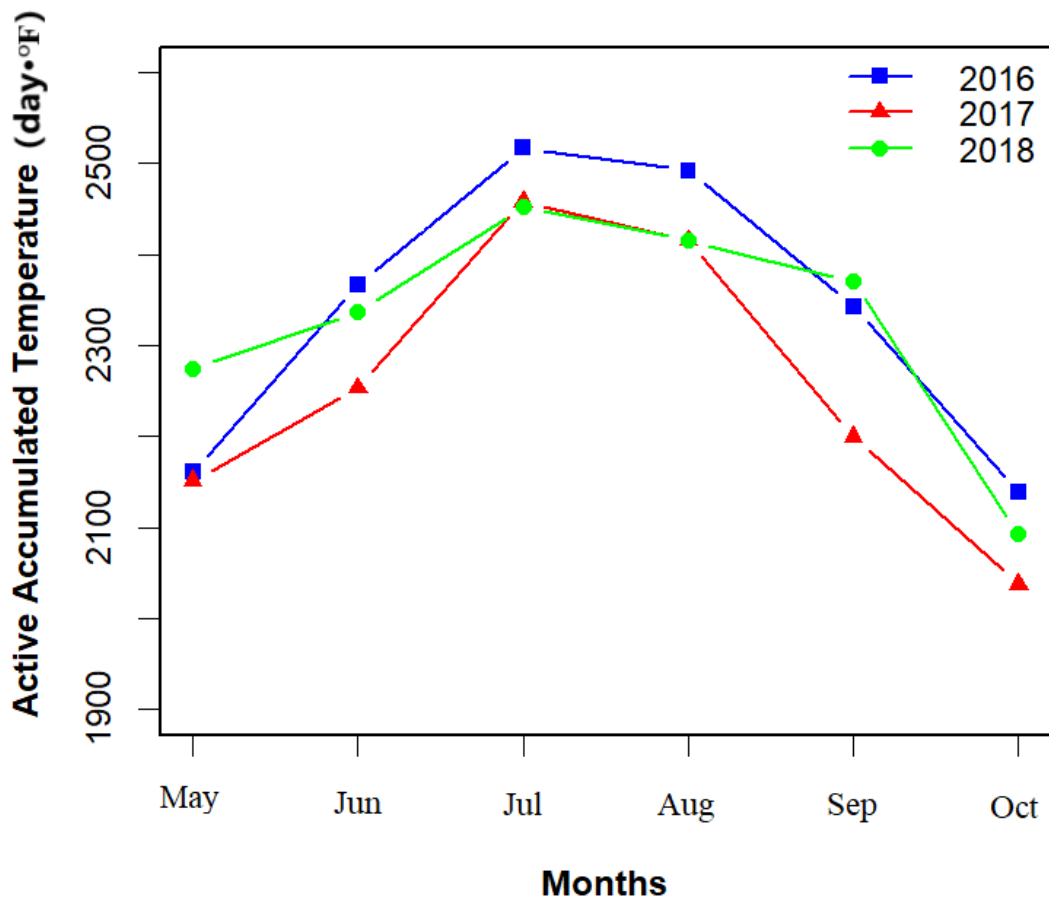


Figure 16. Monthly active accumulated temperature in Auburn University in 2016, 2017 and 2018.

Note: 50 °F was set as the active temperature threshold; the active growing season for turmeric was assumed to be from May to October each year.

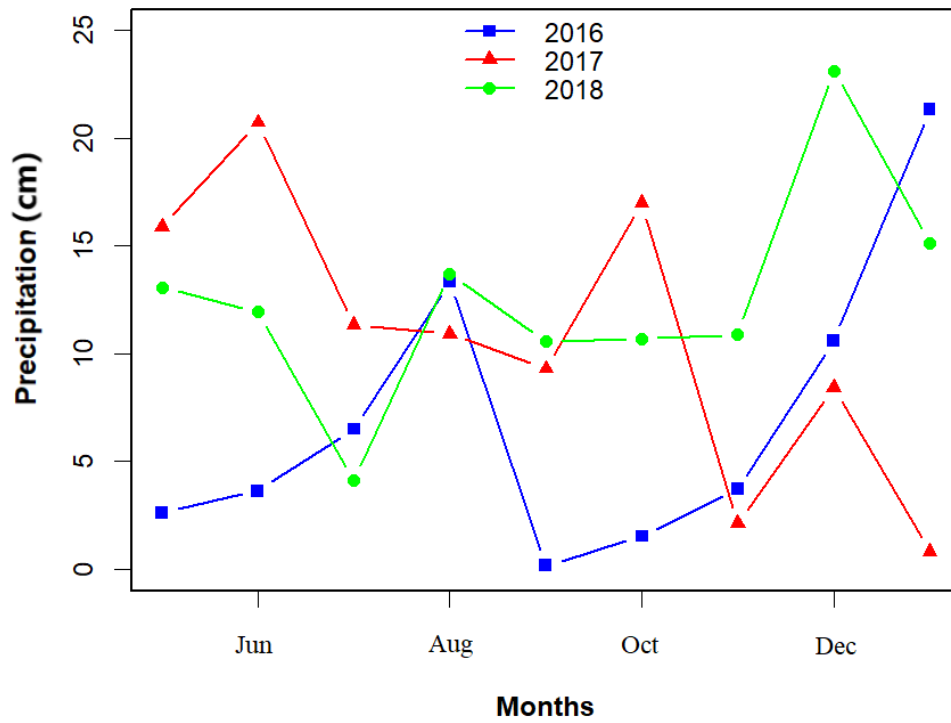


Figure 17. Monthly Precipitation at Auburn University between May through the following January for trials planted in 2016, 2017 and 2018.

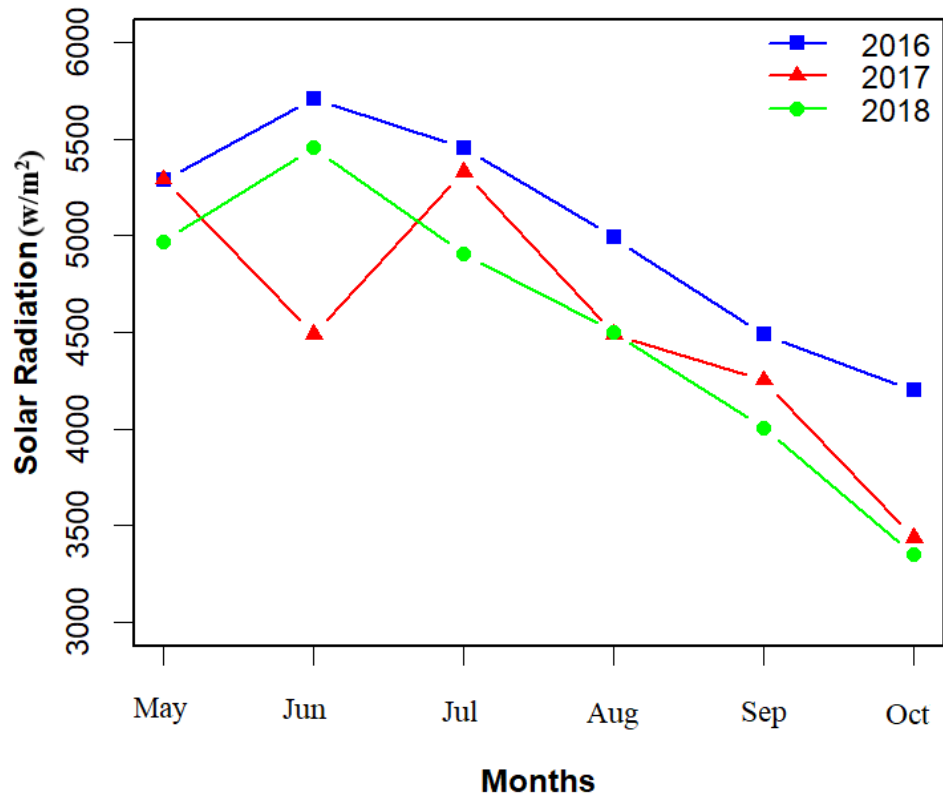


Figure 18. Solar radiation in Auburn University during the active growing season for turmeric from May through October in 2016, 2017 and 2018.

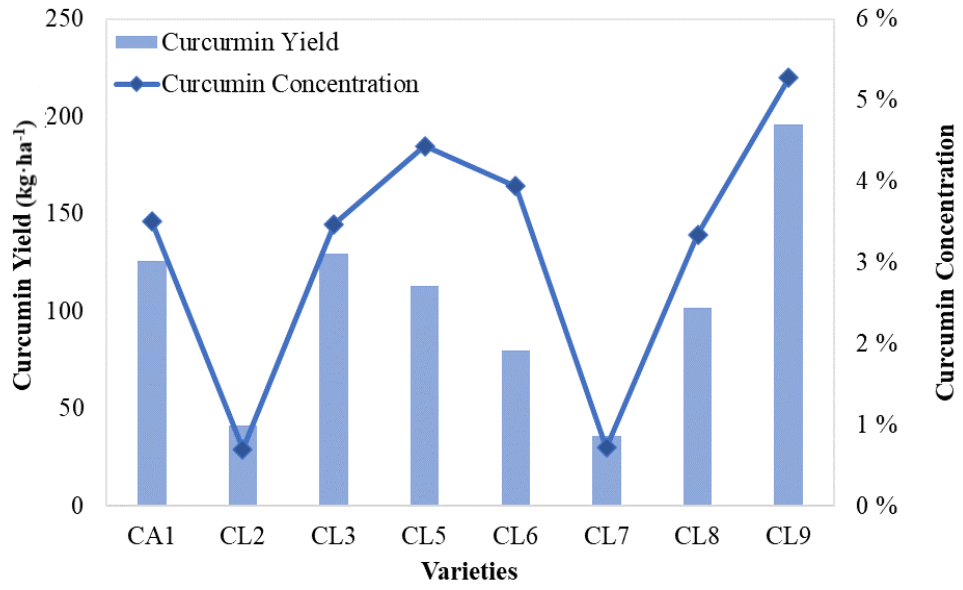


Figure 19. Curcumin yield and curcumin concentration of 8 varieties in 2016. Auburn agronomy field.

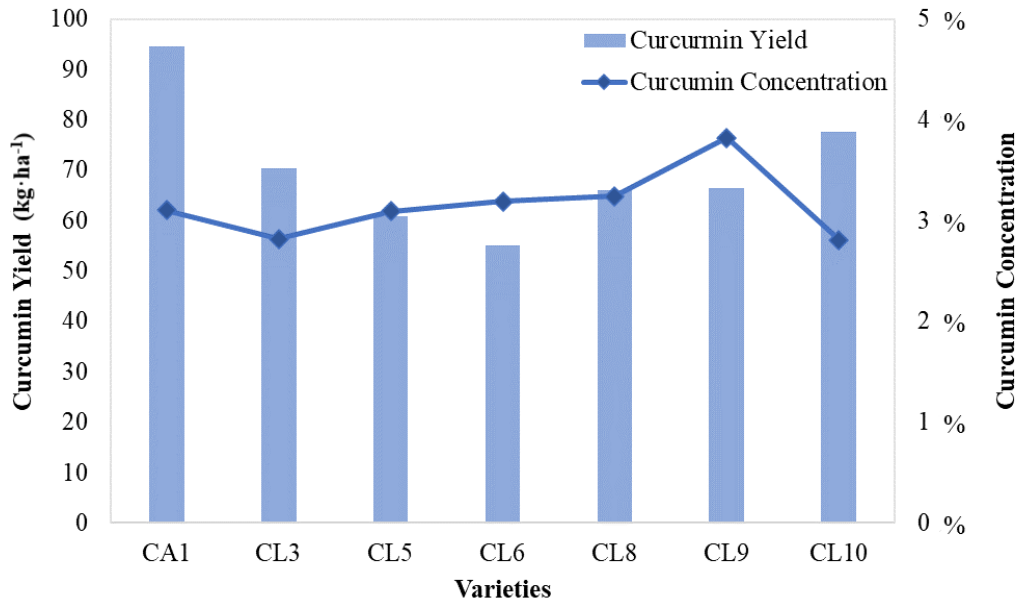


Figure 20. Curcumin yield and curcumin concentration of 7 varieties in 2017. Auburn agronomy field.

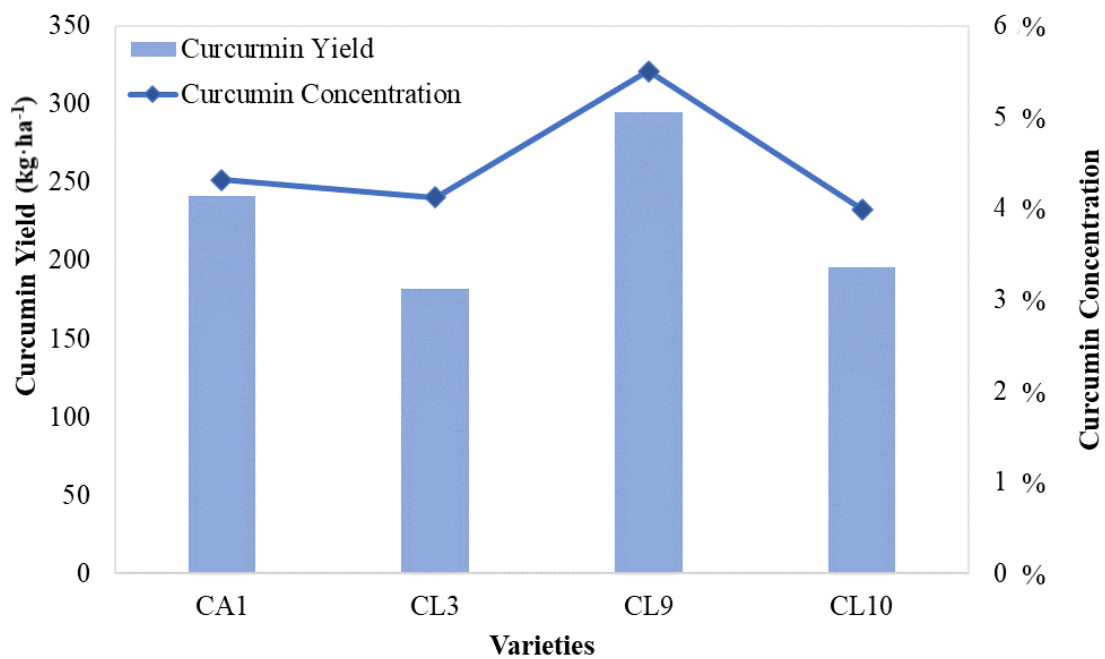


Figure 21. Curcumin yield and curcumin concentration of 4 varieties grown in 2018.

Auburn agronomy field.

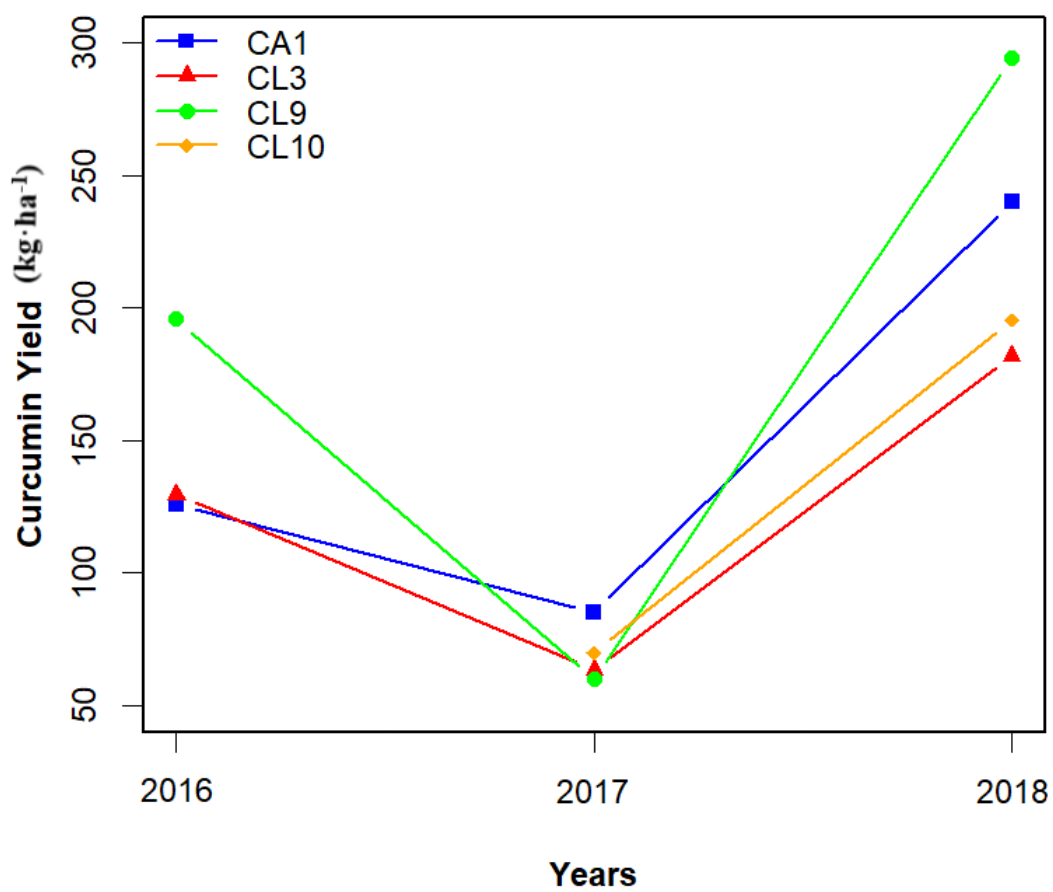


Figure 22. Curcumin yield of 4 varieties in 2016, 2017 and 2018.

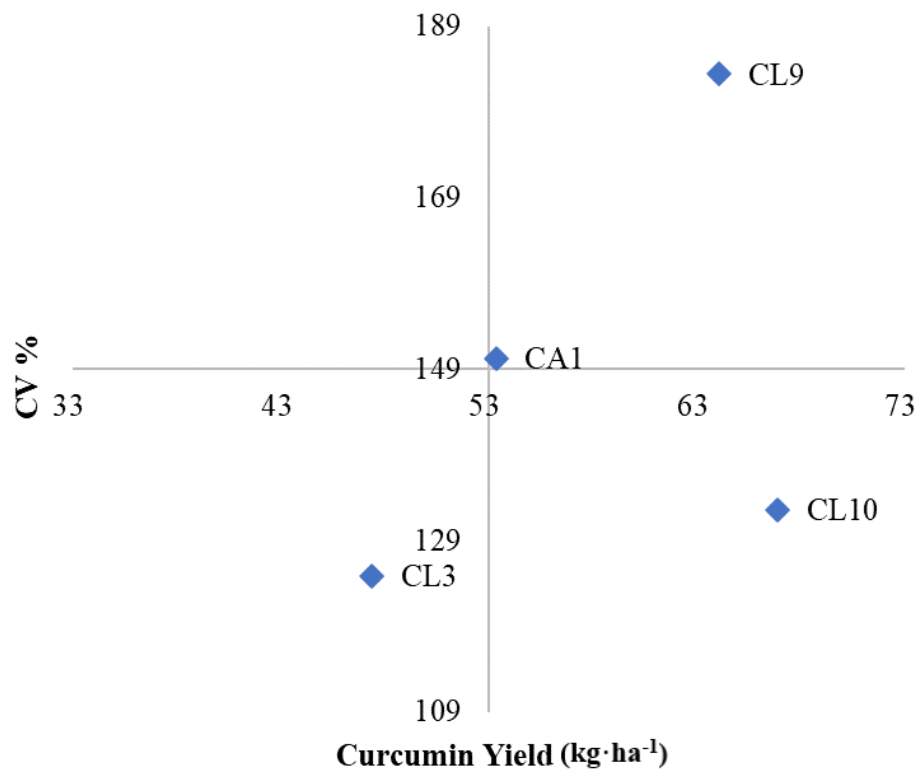


Figure 23. Four-quadrant diagram shows the CV % and curcumin yield of four varieties, annual performance during 2016 and 2018.

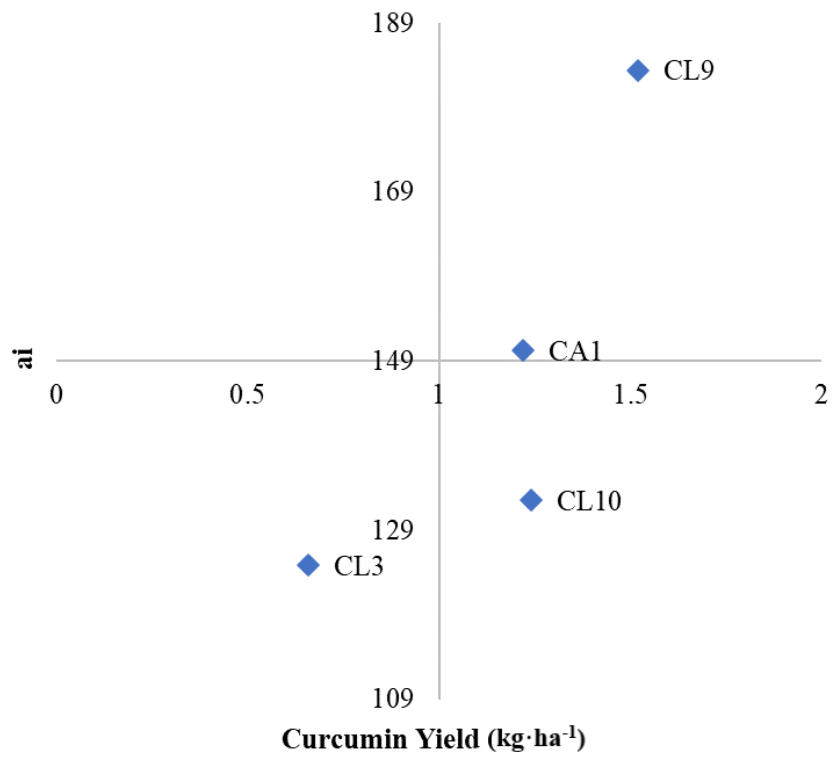


Figure 24. Four-quadrant diagram shows the ai value and curcumin yield of four varieties, annual performance during 2016 and 2018.



Figure 25: Nematodes found in soil samples, collected from turmeric field, Auburn Agronomy Farm, 2019.

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