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Research Articles

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Floral biology and its manipulation for successful breeding programs in cassava (*Manihot esculenta* Crantz) - A review

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Abstract

Cassava is an important staple food of the tropical regions, and it fulfils the dietary requirements of ~900 million people. It is a strategic crop with climate resilience, wide adaptation to diverse climatic conditions and has the potential to cope with the effects of climate change in the future. Cassava is a cross-pollinated crop, pollinated mainly by honey bees. Speed breeding incassava is constrained by its high levels of genetic heterozygosity, sparsely flowering nature of genotypes with high breeding value and low seed set as well as seed germination. In India, cassava breeding was successfully carried out during the last five decades through selection of profusely flowering genotypes, manipulation of flowering in sparsely flowering genotypes using mechanical methods and hormonal treatment. There is variation in flowering time (from 4 months to >eight months) and the number of flowers among genotypes. Different genotype-environment interaction existed on flowering among varieties and a strong environmental effect on the number of flowering peaks within the same variety was noticed. Induction of flowering in erratic flowering types was done through pruning/de-topping of non-branching types and by grafting on to profusely flowering clones. Cryopreservation of pollen of sparsely flowering genotypes was successfully undertaken to maximise the genetic recombination in cassava. The present paper summarises the floral behaviour of cassava in different mating systems and artificial induction of flowering and cryopreservation of pollen that was successfully used to develop hybrids, inbreds and triploids in cassava.

Keywords: Cassava, flowering, Growth regulators, Grafting, Polyploidy, Cryopreservation

Introduction

Cassava (*Manihot esculenta* Crantz) is one of the most important starch-yielding crops grown in many parts of Asia, Africa and South America. It is grown throughout the tropics for its tuberous roots, from which cassava flour, starch, sago, biofuel, bioplastic, biopesticides, etc. are produced. Cassava is the sixth most important source of calories in the human diet. This crop is an essential carbohydrate source for humans and animals, having higher energy production than other root crops, 610 kJ 100 g^{-1} fresh weight (FAO, 2017). Cassava is cultivated in about 224 countries on 26.64 million hectares worldwide, with an annual production of about 296.86 million tons (FAO, 2017). In India, it is cultivated in 1,99,000 ha with a yearly production of 41,71,000 tonnes.

Cassava is a native to South America and the Southern and Western Mexico. It is one of the first crops to be domesticated, and there is evidence that it was grown in

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Peru about 4000 years ago and in Mexico about 2000 years ago (Duke et al., 1983). It has been generally believed that cassava originated from Brazil and Paraguay (Olsen and Schaal, 1999). It spread throughout Central and tropical South America and was taken by the Portuguese to Africa in the 16th century. Portuguese travellers from Brazil introduced cassava to India in northern Kerala during the 17th century. Later, cassava became important as food, feed and for industrial use in India and other Asian countries.

Cassava is grown throughout the tropical regions between latitudes 30° N and 30° S and is potentially one of the most efficient carbohydrate producing crops. It is adapted to temperatures ranging from 18°C to 25°C, rainfall of 50 to 5000 mm annually, and poor soils with pH ranging from 4 to 9. It is an erect growing shrub, branched or un-branched, with palmate partite leaves. The improved varieties were mainly developed through hybridization, followed by clonal selection.

In this review, we describe the floral biology of cassava and its variation in different mating systems. Various approaches adopted to manipulate the flowering for accelerating cassava breeding programs are discussed in detail. The paper also focuses on strategies that involve regulating floral transitions through hormonal application, gene expression and cryopreservation of pollen for long-term use.

Floral biology

Cassava is monoecious, bearing separate male (staminate) and female (pistillate) flowers on the same plant. The inflorescence develops at the reproductive branches, rarely in the leaf axils on the upper part of the plant. The flowers have an indefinite structure called perianth or perigonium, consisting of five cream, yellow, greenish, reddish or purple tepals. The male flower is generally half the size of the female flower with a thin, straight and short pedicel, while the female flower is thick, curved and long. The basal disk of the male flower is divided into ten lobes, and ten stamens originate between them. Stamens with anthers are arranged in two whorls. The five external stamens are separated, longer than the inner ones, and join together on the top to form a set of anthers. The female flower has a ten-lobed basal disk, less lobulated than the male flower. The ovary is tricarpellary with six ridges mounted on the basal disk. The three locules contain one ovule each. A very small style is located on top of the ovary and stigma with three undulated, fleshy lobes (Alves, 2002).

In cassava, flowers are borne in a single branched panicle, with female flowers at the base and male flowers clustered towards the tip (Fig. 1a,b). The male flowers are more numerous than female flowers (Fukuda et al., 2002). Flowers usually begin to open around 10 AM and remain open for about one day (Ceballos et al., 2002). Cassava exhibits protogyny, and on a given branch, female flowers open first, and the male flowers open 10-15 days later. When male flowers open, the female flowers on the same branch are already fertilized or aborted. However, flowering on a single plant may last for more than two months, and both self- and sib-fertilization may occur, with the proportion of each dependent on the genotype, the environment, and the presence of pollinating insects (Kawano, 1980; Jennings and Iglesias, 2002). No genetic or physiological barrier prevents self-pollination in cassava. The intense endogamic depression and vegetative propagation act as a biological mechanism by which the high rate of heterozygosity of the species is maintained (Kawano et al., 1978; Kawano,1982).

Cassava is an outbreeding species possessing 2n=36 chromosomes and is considered an amphidiploid or sequential allopolyploid. In cassava, detailed karyomorphological studies showed haploid chromosomal complement with three functional nucleolar chromosomes. Six chromosomal types in duplicate suggest the allopolyploid nature of present-day cultivated types (Jos and Vasudevan,1989). Triploid (2n=54) and (2n=72) hybrids are also available in cassava. The meiosis in pollen and megaspore mother cells recorded regular bivalent formation with no abnormalities (Graner, 1935). Selection for high yield of tuberous roots and ideal non-branching plant type during domestication reduces seed fertility.

In cassava, the time and frequency of flowering are under genetic control and are strongly influenced by environmental factors like altitude and plant branching habit. For some clones, induction of flowering depends on long photoperiods up to 16-hour day length and temperatures of around 24°C (Keating, 1982; Alves, 2002). Cassava varieties exhibited wide variation in their flowering behaviour, which is positively correlated with the branching pattern of the genotype. Based on the flowering habit, the varieties are classified into non-flowering, sparsely flowering, moderate flowering, and profusely flowering genotypes. Generally, erect non-branching genotypes with high harvest index were selected as commercial varieties. However, the non-flowering nature of these genotypes limits their further utilization in breeding programmes. Hence, parents with a profuse flowering nature and extended periods of flowering were selected for initiating new cassava breeding programmes.

Fertility

The cassava pollen is generally yellow or orange and is large compared to other flowering plants. The pollen grains of cassava are quite large (90-150 micros) with 20-60% germination. The pollen size differs based on the ploidy level of the genotype. Cassava pollen loses

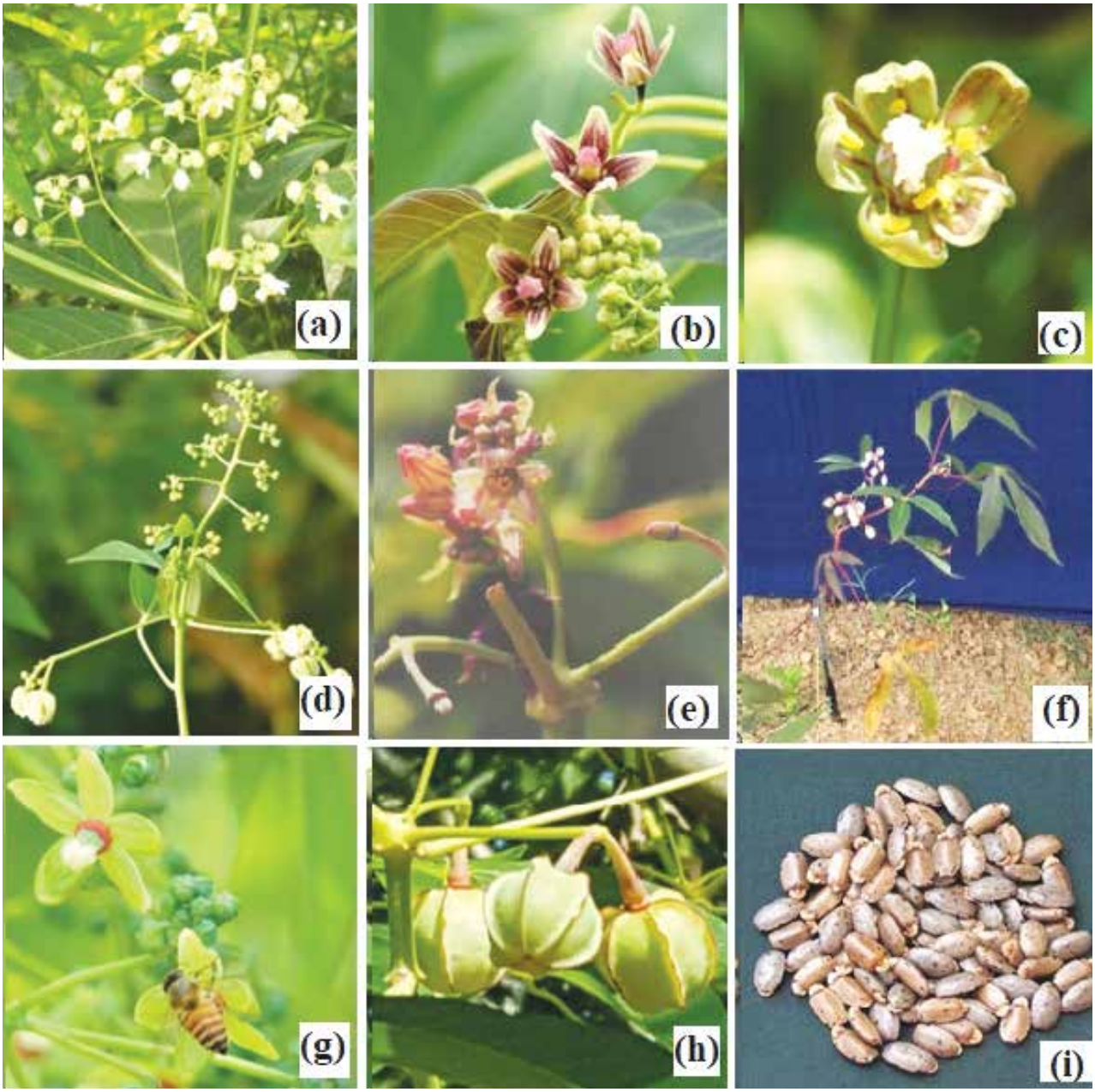

Fig. 1. Floral morphology of cassava: (a) Male flowers, (b) Female flowers, (c) Bisexual flower, (d) Male sterility, (e) Flowers of interspecific hybrids (*Manihot esculenta* X *M.caerulescens*), (f) Early flowering in inbreds, (g) Pollination of flowers by honeybees, (h) Fruits and (i) Seeds

viability rapidly after it is shed. Insects, mainly honey bees, generally cross-pollinate cassava (Fig.1g). The seed-setting ability of the cassava varieties has been reduced due to continuous vegetative propagation and directed selection for non-branching types. Fertility was highly variable, and the pollen's ability to promote seed set was unrelated to the fertility of the variety's female flowers (Jennings, 1962). Male sterility is common in cassava and among many varieties studied, 20 percent had deformed anthers and were male sterile (Cours, 1951). Pachytene pairing in relation to pollen fertility was studied in cassava clones with different levels of pollen fertility. Highly pollen-fertile clones showed regular pachytene and meiotic divisions. In the partially pollen-fertile clones, different pachytene abnormalities like non-pairing, deletion, duplication, and inversion were found in different chromosomes. The extent of pachytene abnormalities indicates the degree of cryptic structural hybridity in the cassava genomes (Jos and Vasudevan,1989).

Male and female cyathia have distinct developmental phases in cassava (Perera et al., 2012). Pollen viability was high during immature stages of plant development, and pollen mortality was common at later stages. Pollen

trimorphism in male gametophytes contributes to larger or smaller pollen size (Perera et al., 2012). The fruit and seed set were drastically reduced when flowers were covered for 2 or 3 days after anthesis day. The pollen tube growth rate is fast during the first 6 hours after pollination. The growth slows down after that, taking ten additional hours to reach the end of the beak. Although several tubes may reach the nucellar beak, only one was observed entering the embryo sac (Ramos et al., 2019). Genotypic and environmental factors and the manual manipulation of inflorescences and cyathia influence the timeline of fertilization. In cassava,early embryo rescue and ovule culture at 7 to 42 days after anthesis resulted in plants with increased levels of homozygosity up to 85.7%.

There is differential genotype-environment interaction on flowering among varieties, as well as a strong environmental effect on the number of flowering peaks within the same variety. Flowering (up to four blooms) was found to be associated with branching. Most flower buds formed at the early growth were found to be abortive. There is variation in flowering time (from four months to >eight months) and in the number of flowers between varieties. Late pollination enhanced seed set in less responsive clones. The uninterrupted availability of soil moisture and cool climate favoured early flowering and fruit set in cassava varieties (Ravi, 2005). Weather conditions influence flower opening; cloudy weather delays the opening of flowers, and capsules mature in 75 to 90 days (Fig.1a&b).

Self-pollination resulted in a low seed set of 33.15% against >80% in cross-pollination. Inbreeding led to the induction of bisexual flowers, male sterility, cleistogamy, and other floral variations in cassava (Fig.1c,d,e). Hybridization of cassava with wild species (*Manihot anomala* Pohl., *M.oligantha* Pav ubsp. Nesteli, *M. gracilis* Pax and *M. zehntneri* Ule) were fertile. Meiosis of the four species was regular with 18 bivalents and equal distribution of the 18 chromosomes during Anaphase I at each pole without any laggards, restitution nuclei or polyads (Nassar, 1978.). In interspecific hybrids of cassava with cera rubber, the random transmission of some of the parental chromosomal types through the male gametes of the interspecific hybrid was reported based on a karyotypic study (Magoon et al., 1971). The interspecific hybrids of cassava with *Manihot caerulescens* have bigger flowers (Fig.1f) and were found to be profusely flowering (Sheela et al., 2002). The fruit of cassava is an ovoid to ellipsoid, septicidal capsule (Oliveira and Oliveira, 2009), which dehisces two to three months after fertilization (Halsey et al., 2008). The seeds are carunculate, with abundant endosperm and an embryo with thin, flat cotyledons (Orlandini and Lima, 2014).

Polyploidy

In cassava, triploids recorded higher yield and starch content than diploids. Polyploidy seems to affect floral traits associated with premating barriers between polyploids and diploids (Pegoraro et al., 2016; Porturas et al., 2019). An increase in the length of pedicels accompanied the change from the diploid condition to the tetraploid condition. However, the initiation of flowering in tetraploids was delayed compared to the diploid counterpart. The triploids and tetraploids have larger flowers than the diploids. There was a significant reduction in pollen fertility and seed setting among the induced tetraploids compared to their diploid progenitors. The induced tetraploid showed irregular meiosis. At Metaphase-1 univalents, trivalents and quadrivalents were observed in addition to bivalents. In tetraploids, pollen sterility was very high (62-78%), presumably due to irregular meiosis, and the seed set was low. Triploids were produced by crossing diploids with the induced tetraploids(Sreekumari et al., 2000). The fruit set was comparatively high (20.5-34.3%)when diploids were used as the female parent. The pollen grains were highly sterile (>95.8%) in most triploid clones, ranging from 8.5 to 12.6%. Micropollen, a characteristic feature of triploids, is common. Fruit set and seed set were meagre when triploids were used as female parents. The occurrence of natural triploidization events has been reported (Sardos et al., 2009; Pillai et al., 2003) and it may also play a role in the evolution of new land races in cassava.

Manipulation of fertility

Cassava genotypes exhibited tremendous variation in their flowering and can be manipulated through mechanical/ exogenous application of plant growth hormones. The wild relatives of cassava are generally propagated through seeds. The highly fertile interspecific hybrids of cassava with closely related *Manihot* sp could be used as a donor parent in backcross breeding programmes to transfer seed fertility to the cassava varieties with reduced pollen and ovule fertility (Jennings, 1962).

Grafting/Pruning

The grafting technique is a viable tool for floral induction since it can promote the movement of mobile elements throughout the plant, such as water, nutrients, metabolites, and proteins (Mudge et al., 2009). It can transfer floral stimulus between different cassava genotypes. The initiation of floral development starts with the movement of florigen (signal), produced in the leaves and transported to the apical meristem through the phloem, where the interaction with other factors occurs (Amasino, 2010; Ceballos et al., 2017). The use of profusely flowering rootstocks could transfer the flowering stimulus to scions of non/sparsely flowering genotypes. Grafting in the early stages of plant

development increases survival and also favours the induction of flowering. High fruit set was found in less flowering genotypes when grafted onto highly flowering ones. An increase of 201% in the production of male flowers, 560% of female flowers, and 400% of fruits in BRS Formosa, a low flowering genotype grafted on a profusely flowering genotype, BGM0823 was reported. The grafted cassava plants recorded an increase in shoot production, although there was no change in the fresh root yield. Hence, the grafting of genotypes with high flowering rates can induce flowering in genotypes with low flowering rates (Souza et al., 2018). The effects of grafting have a genotypic dependency, which limits the potential for its generalised use for enhancing flowering in crossing nurseries in cassava breeding programs (Ceballos et al., 2017). Budding of sparsely flowering plants on profusely flowering genotypes also resulted in the induction of flowering in sparsely flowering cassava genotypes.

Pineda et al., (2020) reported that pruning young branches prevents the abortion of the first inflorescence, fostering seed production much earlier than in untreated plants of late flowering cassava genotypes. They advocated the combined use of extended photoperiod, pruning, and BA application to enhance flowering of late flowering genotypes such as CM 4919-1 and SM 3348-29, which otherwise produce very little or no seed. Besides, detopping of the shoot (30 cm from the top) of non-branching genotypes resulted in the induction of flowering in several low flowering cassava genotypes in India (9S174, Sree Jaya) of cassava (Darshan, 2017).

Plant growth regulators

The shift from the vegetative to the reproductive stage depends on both endogenous (hormones) and environmental (temperature and photoperiod) signalling for the differentiation of the apical meristem into floral meristem (Bernier and Périlleux, 2005). This change in the apical meristem can be induced by the exogenous application of growth regulators such as auxins, gibberellins, abscisic acid, and ethylene (Yang et al., 2016). The effect of plant growth regulators (PGRs) to regulate plant reproductive development has been researched in many plant species (Rademacher, 2015).

Indira et al., (1977) attempted exogenous application of growth regulators to induce flowering in cassava. Indole acetic acid (IAA), Naphthalene acetic acid (NAA), and ascorbic acid (AA) had a positive effect in promoting flowering in cassava as compared to other growth regulators like TIBA, Ethrel, and Kinetin. IAA (50ppm) and AA (100ppm) treated plants of a shy flowering landrace S1315 resulted in a higher number of female flowers and a higher fruit setting percentage. Application of ascorbic acid @100 ppm resulted in the initiation of flowering in the fifth month, followed by treatment with NAA @ 50 ppm. Ascorbic acid @ 100 ppm induced a higher number of flowers in sparsely flowering cassava varieties. The nutritional conditions affect cassava flowering behaviour, and flower/fruit production was significantly enhanced in the unfertilized (NPK) treatment across four different genotypes (Pellet and El-Sharkawy,1993). Induction of flowering *in vitro* plants by adding growth regulators *viz.*, gibberellins and cytokinin in the presence of auxin has also been reported (Tang et al.,1983).

The ethylene signalling affects the floral development in cassava. The effect of the anti-ethylene plant growth regulator, silver thiosulfate (STS), on mitigation of ethylene's effects on cassava flower development was reported. STS did not affect the timing of flower initiation but enhanced the development of early inflorescence, flower development, flower longevity and increased flower numbers (Hyde et al., 2019). Studies on silver accumulation and treatment localization support the hypothesis that the beneficial effects of STS are confined to tissues of the shoot apex. The most effective timing of application was before inflorescence appearance.The additional spraying of benzyl adenine in pruned plants also promotes flower development and often results in the feminization of male flowers (Pineda et al.,2020).

Photoperiod

Pineda et al., (2020) studied the effect of extended photoperiod on flowering of cassava varieties. Five genotypes with contrasting flowering behaviour were grown in dark and extended photoperiod conditions for three seasons. Extended photoperiod was achieved with different red light-emitting diodes (LEDs) with 62-635 nm wavelength all night long or through night breaks. Extended photoperiod reduced height and number of days to first branching, particularly in non- or late-flowering genotypes. They reported that a minimum of 0.02 $\mu molm^{-2}s^{-1}$ was required to induce earlier flowering in plants illuminated all night. Extended photoperiod promoted earlier flowering in erect-plant genotypes of cassava. They recommended the installation of 50W LED lamps, fixed at 3 m above ground in a 4.5 m grid in pollination nurseries to extend photoperiod in cassava for accelerating the breeding programmes.

Expression of floral genes in cassava

Studies on the model plant *Arabidopsis* have helped to understand the regulatory role of various gene networks involved in floral transition in plants (Srikanth and Schmid, 2011; Wellmer et al., 2014; Chen et al., 2018). The genes *CONSTANS (CO), FLAVIN KELCH F BOX 1 (FKF1), FLOWERING LOCUS T (FT)* and *GIGANTEA (GI)* have key regulatory role in determining floral transition in *Arabidopsis* (Fowler et al., 1999; Fowler et al., 1999; Andrés and Coupland, 2012). In many plant species, flowering is induced by the synthesis of the mobile

protein, *FT* in leaves (Andrés and Coupland, 2012; Chen et al., 2018). The *FT* locus is highly conserved among the flowering plants. Thus, overexpression of *Arabidopsis AtFT* in cassava induces early flowering within four to five months of planting in the transgenic cassava lines (Bull et al., 2017). Pollination of pistillate and staminate flowers from clonal propagates resulted in the development of viable seeds in cassava (Bull et al., 2017). Developing cassava with heritable allelic edits but lacking foreign DNA ie the T-DNA encoding the genome editing tools, requires the generation of a segregating population in which the T-DNA can be crossed out. Cassava seldom flowers in glasshouse conditions, but expression of *AtFT* in the glasshouse-grown transgenic cassava permitted rapid flowering and heritability of edited lines (Bull et al., 2018). This shows the potential scope of including genetic engineering technology in speeding up floral transition in cassava breeding programs. The cassava genome encodes for two *FT* genes *MeFT1* (Manes.12G001600.1) and *MeFT2* (Manes.13G000800.1) (Adeyemo et al., 2019). Expression studies showed the modulation of the expression pattern of MeFT2 in response to photoperiod (Adeyemo et al., 2019). Both *MeFT1* and *MeFT2* genes displayed higher expression in early flowering cultivars, suggesting their functional role in regulating flowering in cassava (Adeyemo et al., 2019). Further studies on the functional characterization of *MeFT1* and *MeFT2* would help to understand the molecular mechanism of the genes for manipulation through CRISPR-mediated breeding.

Behnam et al., (2021) found that cassava shares conserved genes for the photoperiodic flowering pathway, including florigen, anti-florigen and its associated transcription factor (*GIGANTEA, CONSTANS*, *FLOWERING LOCUS T*, *CENTRORADIALIS/TERMINAL FLOWER1* and *FD*) and florigen downstream genes (*SUPRESSOR OF OVEREXPRESSION OF CONSTANS*1 and *APETALA1/ FRUITFUL*). In cassava, flowering was induced in the dry season in the mountain regions, and flowering time was found to be correlated with the expression of *MeFT1*, and homologs of *Arabidopsis GI*, *PHYA*, and *NF-Y*s (Tokunaga et al., 2022) and can be a survival mechanism during moisture stress.

Cryo-conservation of cassava pollen

As described by Engelmann (2004), cryopreservation is the conservation of live tissues at hyper-low temperature (-196°C), mostly that of liquid nitrogen, which is an important technique currently available to guarantee the safe, cost-effective, and long-term conservation of germplasm. All metabolic processes and cell divisions are arrested at this condition (-196°C). Hence, the live materials stored in this condition can be conserved without any change for a hypothetically limitless period of time.

The cryo conservation of pollen can potentially overcome challenges of breeding programs, such as flowering asynchrony between different parent genotypes, and the production of insufficient pollen in nature. Cryopreservation of pollen grains to conserve nuclear genetic diversity is desirable in horticultural crops for various reasons. Pollen grains, which are cryopreserved can be a significant approach for the crossing of elite plants particularly in crops where flowering is difficult, production of hybrid seeds, development of pre-breeding lines, germplasm exchange, biotechnological and other basic studies. The objective of the development of a protocol for pollen cryopreservation is to collect matured viable pollen from desirable plants and treat it to retain its viability, germination ability, and ability to fertilize the ovule in natural condition after storing them in liquid nitrogen (Hanna and Towill, 1995). Cryopreserved pollen allows plant breeders to plan hybridization programmes across geographical and seasonal limitations (Ganeshan et al., 2007). Pollen cryobank ensures year-round availability of pollen for biology, biotechnology, and other research programmes (Shivanna, 2003). Cryopreserved pollen grains also serve as conservation of genetic diversity where it is difficult to preserve diversity as live plants and seeds.

The crossing among the lines/varieties of cassava and later selection (mass selection) based on phenotype to identify elite plants within the F_1 progenies and subsequent clonal generations is the widely applied method of developing new varieties in cassava (Ceballos et al., 2004). The hybridisation between the elite lines is very difficult in cassava since the flowering is dependent on environmental conditions and the genotype. Lack of synchronization of flowering between the parents is the major drawback in cassava breeding. This type of flowering behaviour slows down cassava improvement through breeding. In such contexts, it is convenient to store pollen from the desired male parent for later use in hybridisation with the female parent when the female flowers are available. The low fertilization rate in cassava could be due to the low pollen viability reported for *Manihot* species, because pollen lasts for only 48 hours after anthesis (Halsey et al., 2008). According to Vieira et al., (2012), the pollen remains viable for six days when conserved in calcium chloride, and loses its viability when stored fresh at room temperature. Thus, the difficulties in the breeding of cassava can be overcome by the use of stored pollen.

The detailed protocols for pollen cryopreservation of cassava have been given by Vivek et al., (2019a). The male buds were bagged one day before anthesis and collected on the next day morning between 09.00 AM and 10.30 AM. Freshly collected male flowers were placed in a petri dish lined with moist paper and brought immediately to the laboratory. The collected flowers were stored in liquid nitrogen after placing them in the cryovials.

The stored cassava pollen samples were tested for viability using acetocarmine staining, *in vitro* germination tests, andwas also used for hand pollination. In the staining test, pollen grains were stained with acetocarmine and observed under the microscope. Deeply stained pollen grains were classified as viable, while those with abnormal size and stained in a light colour were considered non-viable. A non-significant difference was observed between the varieties for the viability of fresh pollen in the acetocarmine staining test. In contrast, a significant difference was observed for *in vitro* pollen germination and fruit set in the field conditions. Similarly, Dutta et al., (2013) reported that the *in vitro* pollen germination test was reliable in the mango pollen viability study, whereas acetocarmine tests obtained overestimated results. Chaudhury et al., (2010) and Shivanna and Helpson-Harrison (1981) also observed the over estimation of viability in fresh and stored mango pollen.

Medium for pollen germination under *in vitro* conditions was standardised in several crops like mango, citrus, gladiolus, rose, tomato (Ganeshan et al., 2007), and taro (Mukherjee et al., 2016). Germination of cassava pollen under *in vitro* conditions was achieved using the sitting drop method. A drop of pollen germination medium containing 5% sucrose, 300 mgl^{-1} calcium nitrate, 200 mgl^{-1} magnesium sulfate, 100 mgl^{-1} boric acid, and 100 mgl^{-1} potassium nitrate (Mary et al., 2015) was laid on the microscopic slide and pollen was brushed. This was incubated at room temperature for six hours, and through an optical microscope, germinated pollen grains were recorded. Stored pollen was used to test their fertility/crossability for controlled field pollination. The hand pollination was done by placing cryopreserved pollen onto the receptive stigma and pollinated female flowers were immediately protected from insects with muslin cloth. Cryopreserved cassava pollen for 45 days recorded 63.9% and 59.1% staining as well as 51.0% and 49.5% *in vitro* germination in Vellayani Hraswa and Sree Padmanabha (Fig. 2) and the fruit set recorded was 47% and 46.7%, (Fig. 3) respectively (Vivek et al., 2019b). Both staining percentage and *in vitro* pollen germination were reduced to zero at later stages of storage. Cassava pollen is highly susceptible to desiccation, and there is rapid moisture loss and reduction in viability when stored at room temperature. Vieira et al., (2015) kept the cassava pollen at room temperature and observed total loss of pollen viability after 24 hours. Daniel (2011) observed poor pollen survival in West African yams with drying and suggested the 'wet-cold' storage procedure.

The pollen once stored at a temperature below -160°C would theoretically have an infinite period of longevity (Stanwood, 1985). Mukherjee et al., (2016) studied pollen cryopreservation in taro and observed that the degree of successful fruit set in taro with cryo-stored pollen was on par with conventional fruit set. In wild

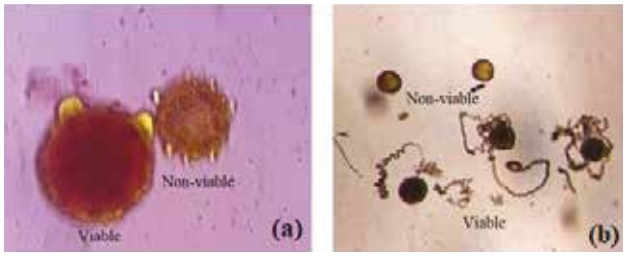

Fig. 2. Viability of cryopreserved cassava pollen assessed by acetocarmine test and *in vitro* germination tests (a) pollen staining and (b) germinating pollen under *in vitro* condition (Source: Vivek et al., 2019a)

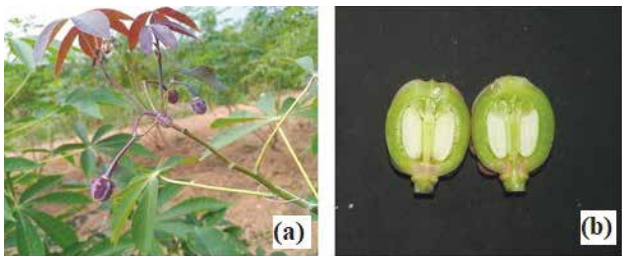

Fig. 3. (a) Fruit set by the cryo-stored pollen and (b) Developing seed pollinated by the cryo-stored pollen

pineapple accessions, Silva et al., (2017) achieved 62.67% *in vitro* pollen germination and 70.83% seed formation with cryopreserved pollen grains. Significantly higher pollen viability of three Indian mango cultivars was reported by Dutta et al., (2013) when stored at -196°C by different pollen viability tests such as acetocarmine, fluorescein diacetate (FDA), and *in vitro* germination. Lora et al., (2006) efficiently stored cherimoya (Annona cherimola Mill.) pollen at −196°C (liquid nitrogen). Similarly, Alba et al., (2011) conserved pollen from 12 olives (*Olea europaea* L.) cultivars for 1 year in liquid nitrogen. The highest mean percentage of fruit set in cassava was obtained when pollination was carried out between 11.30AM to 12 noon. The fruit set percentage was reduced when pollination was carried out before and after the peak receptivity period (11.30AM-12noon) of the stigma (Vivek et al., 2019a). In cassava, many non-branching genotypes with suitable plant type flowered rarely. Even though flowering can be induced through de-topping and hormonal treatment, the number of flowers and flowering peaks will be less. However, the cryopreservation of the rare pollen will help storage and need-based use of pollen in cassava breeding programmes. It also helps in an international cassava pollen exchange, strengthening the collaborative inter-institutional breeding programmes.

In cassava, combining pruning young branches and spraying BA allowed the production of more seeds from erect cassava genotypes in a short period. The implementation of these procedures along with cryo preservation will improve the breeding efficiency in cassava (Pineda et al., 2020).

Breeding success in India

ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India, initiated the cassava breeding programme in 1963. A total of 1216 accessions were conserved in the field gene bank at the National Repository of Tuber Crops germplasm at ICAR-CTCRI, Sreekariyam, Kerala, India. Cassava breeding in India was focused on developing varieties for table purpose as well as for industry and selection parameters were set accordingly. Thirty-three cassava varieties were released in India through different cassava breeding programmes. The developed varieties include short duration (6 months), high starch triploids, nutrient efficient, and cassava mosaic disease-resistant varieties. The improved varieties were generally developed through hybridization, followed by clonal selection. Most of the released varieties released are diploids. Three triploid hybrids, Sree Harsha, Sree Athulya and Sree Apoorva with high extractable starch content (30-34%) were released through a triploidy breeding programme. Recently, cassava mosaic disease (CMD) emerged as the major threat to cassava cultivation in Southeast Asian countries. Five high-yielding varieties *viz*., Sree Reksha, Sree Sakthi, Sree Suvarna, PDP-CMR1 and Sree Kaveri with resistance to CMD caused by Indian Cassava mosaic virus and Sri Lankan cassava mosaic virus were also released in India. All these breeding successes in cassava have been brought through the successful manipulation of flowering and the development of the pre-breeding population in cassava.

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Greater Yam: An overview of its phytochemical profile and potential for ensuring food security

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Abstract

Millions of people in tropical nations rely on cultivated yams (*Dioscorea* spp.) as their main source of nutrition. Even though these underutilized crops are rich in nutrients, there is a dearth of information on them, which impedes their development and sustainability. Greater yam (*D. alata*) plant has medical, pharmacological, cosmetic, and industrial uses in addition to its nutritional value. Their bioactive components, which include anti-inflammatory, antibacterial, and antioxidant properties, have recently drawn more interest in scientific investigations. This analysis aims to highlight the undervalued benefits of yams for pharmacological applications and food security. These species differ greatly in their morphology with regard to the length of growing season, the types of tubers produced, the dry matter content of the tubers, and the nutritional and chemical components of the tubers. Nonetheless, there is a dearth of knowledge currently available about yams. As a result, we covered information regarding the botanical description, origin and distribution, genetic resource, agronomy, pharmacological properties, nutritional values, molecular and genomic research, and future prospects regarding yams in this review.

Keywords: Yams, Dioscorea, Food security, Phytochemicals, Bioactivity

Introduction

Some crops may not be consumed globally due to their adaptability to limited growing conditions but are staples for a particular region and contribute significantly to food supply with a nutritionally well balanced diet. Despite of having nutritionally rich content, very few information is available on these underutilized crops that hinders their sustainable development. One of such crops is Greater yam (*Dioscorea alata* L.*). Dioscorea alata* is one of the most important staple crops in Austronesian cultures. It is one of various species of yams that were domesticated and cultivated independently in Southeast Asia and New Guinea for their starchy tubers, including the round yam (*Dioscorea bulbifera*), ubi gadong (*Dioscorea hispida*), lesser yam (*Dioscorea esculenta*), Pacific yam (*Dioscorea nummularia*), fiveleaf yam (*Dioscorea pentaphylla*), and pencil yam (*Dioscorea transversa*). Among these, *D. alata* and *D. esculenta*are the only ones regularly cultivated and eaten, while the rest were usually considered as famine food due to their higher levels of the toxin, dioscorin which requires that they should be prepared properly before consumption. *D. alata* is cultivated more widely than *D. esculenta*, largely because of much larger tubers of the former.

D. *alata* and *D. esculenta* were the most suitable for long duration transport in Austronesian ships and were carried through all or most of the range of the Austronesian expansion. *D. alata* in particular, were introduced into the Pacific Islands and New Zealand (Crowther et al., 2016). They were also carried by Austronesian voyagers

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into Madagascar and the Comoros. The tubers contain minerals such as potassium (30000 mg kg^{-1}), phosphorus (9680 mg kg^{-1}), sulphur (3600 mg kg^{-1}), calcium (2040 mg kg^{-1}), iron (395 mg kg^{-1}), manganese (129 mg kg^{-1}), nickel (28.2 mg kg^{-1}), tin 35.2 (mg kg^{-1}), copper (18.5 mg kg^{-1}), zinc (29.4 mg kg^{-1}), rubidium (63.1 mg kg^{-1}), strontium (9.74 mg kg^{-1})(Vincent Lebot *et al.*, 2022). Besides all these elements, several vitamins like Vitamin C (202 mg kg^{-1}), Thiamine (0.20 mg kg^{-1}), Riboflavin (0.29 mg kg^{-1}), Niacin (2.0 mg kg^{-1}), Pantothenic acid (1.135 mg kg^{-1}), Vitamin B6 (0.42 mg kg^{-1}), Vitamin E (4.6 mg kg^{-1}), Vitamin K (3.0 μg kg^{-1}) and several amino acids such as Threonine (0.18 gkg^{-1}), Isoleucine (0.16 gkg^{-1}), Leucine (0.25 gkg^{-1}), Lysine (0.26 gkg^{-1}), Methionine (0.07 g kg^{-1}), Cysteine (0.06 g kg^{-1}), Phenylalanine (0.017 g kg^{-1}), Tyrosine (0.12 g kg^{-1}), Valine (0.22 g kg^{-1}), Arginine (0.37 g kg^{-1}), Histidine (0.19 g kg^{-1}), Alanine (0.20 g kg^{-1}), Aspartic acid (2.0 g Kg), Glutamic acid (0.43 g kg^{-1}), Glycine (0.16 g/ kg^{-1}), Proline (0.25 g kg^{-1}), and Serine (0.25 g kg^{-1}) are also present in Greater yam tuber (*USDA ARS*, 2014; Lim, 2016).

This plant not only has nutritional values but also has medicinal and pharmaceutical as well as cosmetic and industrial values. Its seed has been used in traditional medicine for the treatment of insomnia, skin infection, herpes, and fever. Different parts of the plant possess a number of pharmacological activities like anticancer, antiviral, immune-modulatory, anti-tyrosinase or skin whitening, and photoprotective activities (Arinathan et al., 2009). An average yield of greater yam is reported to be 20-25 t ha^{-1}. These species have extensive morphological variation in plant structure, leaf structure, length of growing period, types of tubers produced, dry matter of content of tubers, and nutritional and chemical constituent of tubers. However, the existing information regarding yams is very limited. Therefore, in this review we described details about the botanical description, origin and distribution, genetic resource, agronomy, molecular and genomic studies, and future prospectusof yams.

Taxonomy

Greater yamis an underutilised tuber crop, and its common name varies across the world. Most commonly it is known as Guyana arrowroot, ten-months yam, water yam, white yam, winged yam, violet yam, or simply yam. In India, this crop is called in different common names such as Ratalu, Kand, Kachil or Indian Purple yam in different states or regions.

Taxonomical position

The taxonomy of the plant is as follows:

Kingdom : Plantae
Clade : Tracheophytes
Clade : Angiosperms
Clade : Monocots
Order : Dioscoreales
Family : Dioscoreaceae
Genus : *Dioscorea*
Species : alata

Botanical description

This is a large climber, which can reach 15 m in height, with quadrangular winged stems, twining is anticlockwise (to the right). The leaves are opposite, variable in size and shape, but essentially ovate to cordate with a deep basal sinus, acuminate. The male flowers are borne on panicles, up to 30 cm long; the female flowers are on small axillary spikes. Few cultivars produce fertile seeds, and most are completely sterile. Bulbils are sometimes formed in leaf axils, but not so freely as with certain other species. The tubers are usually single and show a great deal of variation in size, shape and colour: they are generally cylindrical but may be long and serpentine to almost globular, and are often branched or lobed, or even flattened and fan shaped. Their weight is usually 5-10 kg though special cultivation practices can produce giant tubers of 60 kg or more. The flesh of some cultivars can be pink or even deep reddish-purple and these forms have been classified as *D. purpurea* Roxb. and *D. afropurpurea* Roxb. but this is not generally accepted.

Origin and distribution

D. alata is not known in the wild state but appears to have been developed from native species originating in the Assam-Burma region, by selection from deeper-rooting forms. Subsequently, it was spread through Thailand and Vietnam into the Pacific region, westwards and southwards to India and Malaysia and thence apparently to Madagascar and East Africa, later to be taken by the Portuguese and Spaniards to West Africa, northern South America and the Caribbean; in the eastern Caribbean and in the Pacific it is the most popular species of yam. It is cultivated throughout the tropical world. In India, greater yam reported to be found in the states like Assam, Tamil Nadu, Kerala, Bihar, Odisha, West Bengal, Rajasthan, Gujarat and Maharashtra.

Genetic diversity in greater yam

The exact location of greater yam origin is unknown. It is assumed to have been domesticated about 6000 years ago and is native to Asia-Pacific, however it has never been seen in its natural condition. In *D. alata*, the highest phenotypic heterogeneity was seen in the southern region of Southeast Asia and Melanesia, the species' likely site of origin. *Ex situ* collections of *D. alata* may be found in the South Pacific islands of Papua New Guinea, Fiji, New Caledonia, Solomon Islands, and Vanuatu, with over 1000 varieties. *Ex situ* germplasm

collections have been developed in India, including the most significant collection at ICAR-Central Tuber Crops Research Institute (CTCRI), Kerala, India, which has 431 accessions. Several international collections, notably the CRB-PT (Centre de Ressources Biologiques Plantes Tropicales INRA-CIRAD, Guadeloupe, France) and the IITA (International Institute of Tropical Agriculture, Ibadan, Nigeria), have been assembled, comprising 181 and 772 *D. alata* accessions, respectively.

Using enzymatic markers, Lebot et al., (1998) assessed the diversity of 269 *D. alata* accessions from diverse locations (South Pacific, Asia, Africa, and the Caribbean). The modest polymorphism of the four enzymatic systems, on the other hand, revealed no relationships between zymotype groups and geographic origins, ploidy levels, and/or phenotypic traits of the accessions.Various molecular markers including RAPD, AFLP and SSR have been used to characterize the genetic diversity of *D. alata* collections. Asemota *et al*. have used random amplified polymorphic DNA (RAPD) analysis to characterize eleven cultivars of the five economically most important yam species grown in Jamaica (*Dioscorea alata, D. cayenensis, D. rotundata, D. trifida and D. esculenta*) (Asemota et al., 1995). Arnau et al. used Amplified fragment length polymorphism markers to assess the genetic relatedness between *D. alata* and nine other edible *Dioscorea* (Arnau et al., 2017). These species include *D. abyssinica* Hoch., *D. bulbifera* L., *D. cayenensis*-rotundata Lamk. et Poir., *D. esculenta* Burk., *D. nummularia* Lam., *D. pentaphylla* L., *D. persimilis* Prain. et Burk., *D. transversa* Br. and *D. trifida* L. Four successive studies were conducted with emphasis on the genetic relationship within *D. alata* and among species of the *Enantiophyllum* section from Vanuatu (Malapa et al., 2005). Cardona et al., used seven intersimple sequence repeat (ISSR) markers to describe the genetic diversity of *Dioscorea alata* L. and associated species from Colombia (42 *D. alata* L., 6 *D. bulbifera* L., 3 *D. rotundata* Poir., and 3 *D. trifida* L. f.)(Cardona et al., 2020).

A total of ten microsatellite loci were chosen by Siqueria *et al.*, and nine polymorphic loci were used to analyse 80 *D. alata* accessions from various parts of Brazil (Siqueira et al., 2014). The power discrimination (PD) ranged from 0.15 to 0.91, while the polymorphism information content (PIC) ranged from 0.39 to 0.78. Six of the markers were shown to be transferable between the *D. bulbifera, D. cayenensis, D. rotundata, and D. trifida* species. For phenotypic and genetic diversity, a working collection of yam (*Dioscorea* spp.) was analysed by Adjei et al., which included 53 landraces and seven improved cultivars from four species (*Dioscorea alata* L., *D. cayenensis* Lam., *D. dumetorum* (Kunth), and *D. rotundata* Poir.) (Adjei et al., 2022). The examination included a field assessment of 24 physical features as well as DNA analysis using 32 SSR polymorphism markers. Between-species diversity was higher than within-species diversity, with *D. rotundata* having the most diversity and *D. alata* and *D. cayenensis* having the lowest. *D. rotundata* and *D. cayenensis* were shown to have a close connection based on combined phenotypic and SSR marker data sets, whereas *D. alata* and *D. dumetorum* remained separate species. Genetically, *D. alata* was connected to *D. rotundata* and *D. cayenensis*, while phenotypically, it was similar to *D. dumetorum*. According to the research, cultivars grown from various farmers may have the same name yet vary genetically.

Collection and conservation

Greater yam gives optimum yields at rainfall of 150 cm evenly distributed over 6-7 months though it will perform moderately well on 100 cm. *D. alata* can tolerate poorer soils than most other species of yam, but it responds well to fertilising. In India, FYM at the rate of 25 t ha^{-1} has been recommended. In Barbados, where the crop is frequently grown as a rotation crop with sugar cane which has been fertilised with a 22:0:22 NPK mixture, yields of about 10 t ha^{-1} are normal, but additional fertilising with NPK at the rate of nitrogen 22 kg, phosphorus 25 kg and potassium 57 kg per hectare gave significant and economic increases in yield. Smaller increases were given when phosphorus was omitted. Application should be about 10 weeks after planting, when the plant is completing its dependence upon the parent sets. It is usually cultivated at low or medium elevations but is grown as high as 2700 m in India. Maturity takes 9-10 months on an average, while certain 'early' types may be harvested as early as 6 months. Harvesting is usually done by forking, however due to the size and irregular form of many cultivars' tubers, damage is common, with 20-25 percent of tubers being damaged. Recent improvements in the Caribbean have resulted in the creation of a mechanised harvester and an 8 percent decrease in damaged tubers. Under typical tropical circumstances, storage lasts 4-6 months. Storage is terminated by the breaking of dormancy if the tubers are sound; if sprouts are removed as they emerge, storage may be prolonged to nearly 8 months.

Pests and diseases

In addition to yam beetles and scale insects, the greater yam is attacked by the larvae of three Lepidoptera species: *Loxuraatymnus, Theretranessus*, and *Tagiadesgana*. The first is the most harmful, since the larvae attack the stems after first eating on the leaves, forcing them to snap off. *Scutellonemabradys*, a yam nematode, is also capable of attacking *D. alata*. One of the most troublesome diseases affecting this species is anthracnose caused by *Colletotrichum gloeosporioides*, sometimes in association with other fungi, notably *Botryodiplodia and Fusarium* spp.; crop losses can sometimes amount to 70-80 per cent. Spraying of zineb or ferbam at 10 day intervals is stated to be effective (Winch et al., 1984). Leaf spot, due to *Cercospora* spp., is reported to be serious in Sri Lanka (Hong et al., 2010). In Guadeloupe, crown-gall,

a bacterial condition caused by *Agrobacterium tumefaciens* has been observed. An internal brown spot has caused serious losses in yams exported from Barbados; this has been traced to a virus infection which also leads to considerable reduction in yield (Okon et al., 2022).

Ethno-botanical uses

The word 'ethno-botany' refers to the studies on how people from a specific culture and region use plants. Different *Dioscorea* species plays a remarkable position in the traditional medicines for the treatment of various diseases (Kumar et al., 2017). There are numerous reports available on ethno-medicinal uses worldwide (Sharma and Bastakoti, 2010; Sheikh et al., 2017). In South Asia, the tuber syrup is used to reduce labour pain and to treat various diseases such as colic pain, asthma, cough, rheumatism, and gastric problem (Foster and Duke, 2000). The native people of Southern Thailand use tubers to cure warts (Maneenoon et al., 2008). The tuber is an important component in limiting water loss during pregnancy, and it also helps to alleviate nausea and vomiting. In Bangladesh, the tubers of *D. bulbifera* are used to treat leprosy and tumours, and to heal sore throats in Chinese medicine (Mbiantcha et al., 2011). In Zimbabwe, the tuber is used to cure wounds and sores, and in Cameroon and Madagascar, the bulbils paste is externally applied to boils and wound (Mbiantcha et al., 2011). *D. alata* was utilised by the native tribal populations of Enugu, Nigeria to treat fever (Aiyeloja and Bello, 2006).

Pharmacological uses

Dioscorea species have been reported to have anti-microbial, anti-fungal, antimutagenic, hypoglycaemic, and immunomodulatory effects (Kumar et al., 2017). The extracts of *D. alata* is identified to have antifungal activities on *Botryodiploidia theobromae* (Eleazu et al., 2013). Antimicrobial activities of yams against gram-positive and gram-negative bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus pyogenes, Vibrio cholerae, Salmonella enteric-typhi, Shigella flexneri,* and *Klebsiella pneumoniae* have been reported by several researchers (Kumar et al., 2013). The existence of anticancer components has been demonstrated by the cytotoxicity of *D. alata* extract on human cancer cell lines (Das et al., 2014). *D. alata* (Maithili et al., 2011) has been shown to have anti-diabetic properties and can be used in the treatment of type 2 diabetes. Diosgenin, a chemical component found in *D. alata*, is used to create steroids like dehydroepiandrosterone (Jesus et al., 2016).

Usage as Food

Greater yam is used mainly as a vegetable, similar to potato, and some cultivars can be used to make French fries and chips, claimed to be superior to similar potato products. Although it is the preferred yam in many parts of the tropics, especially by those accustomed to European dietary habits, it is less highly regarded in West Africa, because it is not suitable for the preparation of 'fufu'. In some countries, such as Philippines, Barbados and Puerto Rico, attempts are being made to develop processed products such as yam flakes or powder from surplus supplies of *D. alata* (Anyaogu, 2013). Coloured cultivars have been utilised as a colouring and flavouring agent for ice cream. Secondary and waste products like severely damaged tubers are often fed to pigs. A typical analysis of the edible portion of the tubers is: water 65-73 per cent; protein 1.12-2.78 per cent; fat 0.03-0.27 per cent; carbohydrate 22-29 per cent; fibre 0.65-1.4 per cent; ash 0.67-2.06 per cent. The starch contains a high proportion of large granules with size ranging from 5 to 50 microns have been reported. The starch gelatinization temperature ranges from 69°C to 88°C and the viscosity from 100 to 200 Brabender units. Unlike most other yam species, starch from *D. alata* has a high gel strength. Starch from white-fleshed and purple-fleshed cultivars have similar typical composition averaging: moisture 13.6 per cent; protein 0.14 per cent; ash 0.22 per cent; amylose 21.1 per cent; reducing sugars 0.18 per cent; pH 7.1; iodine value 5.5. Ascorbic acid contents ranging from 4.9 to 8.2 mg100 g^{-1} of edible portion have been reported, while certain cultivars in the South Pacific have been found to contain 6 $mg100^{-1}$ g of carotene. Three anthocyanins have been isolated from *D. alata* var. atropurpurea and rubella and found to be cyanidin glycosides (Anyaogu, 2013).

Nutritional parameters

In comparison to other tropical tuber crops, greater yams are thought to provide a significant quantity of different nutritional components. The yam tuber is said to be an excellent source of key nutritional components such carbohydrates, proteins, fats, vitamins, and minerals, among others (Arinathan et al., 2009; Mohan and Kalidas, 2010).

Proximate composition

Moisture, ash, crude fat, crude protein, crude fibre, and carbohydrate make up the proximate composition, which is very essential in highlighting the food quality (Polycarp et al., 2012). Ghanaian greater yams had a moisture level of 58 to 79 percent (Polycarp et al., 2012), Indian greater yams had a moisture content of 71 to 92 percent (Shanthakumari et al., 2008), and Nepalese yams had a moisture content of 19 to 30 percent (Bhandari et al., 2003). The ash content of the food determines the presence of important dietary minerals and useful for the development of the body (Otegbayo et al., 2018). Greater yam has a lower ash content than other tuber crops like potato and cassava (Bhandari et al., 2003; Otegbayo et al., 2018). Dietary fats aid in the absorption and preservation of tastes during cooking, resulting in

improved food palatability (Otegbayo et al., 2018). Yam has been observed to have a greater dietary fat or lipid content than potato and sweet potato (Otegbayo et al., 2018). According to researchers, yam species have higher dietary fibre than other tuber crops including potatoes, cassava, and sweet potatoes (Baah et al., 2009; Shanthakumari et al., 2008) as high fibre in the diet aids in the digestion and absorption of nutrients in the large intestine, preventing constipation (Baah et al., 2009). The texture quality of yam is also influenced by non-starchy carbohydrates such as lignin, cellulose, and hemicelluloses (Otegbayo et al., 2018).

In all living species, protein aids in the structural and functional activities of cells, as well as the regulation of metabolic processes. The protein content of yam species is said to be greater than that of other major tuber crops such as cassava (Charles et al., 2005) and sweet potato (Moongngarm, 2013). The crude protein content is reported in Ethiopian yam (*D. bulbifera*) (9.7% dry wt. basis) (Tamiru et al., 2008); Sri Lankan yams (*D. alata and D. esculenta*) (10.16%) (Senanayake et al., 2012); and several Indian varieties (*D. alata, D. bulbifera, D. esculenta, D. oppositifolia, D. pentaphylla*(Aprianita et al., 2014). Carbohydrate is a component of the proximate composition of food that gives energy to the body and is crucial to the construction and operation of cellular mechanisms (Baah et al., 2009). The sugar and starch contentsin yams are said to be lower than that of potatoes and cassava (Baah et al., 2009; Ohene Afoakwa and Simpson Budu, 2012; Otegbayo et al., 2018). Greater yams, followed by cassava (20%), taro (4%) and sweet potato (2%) are said to offer 12 % of the energy diet for humans in tropical nations (Otegbayo et al., 2018).

Vitamins

Different dietary vitamins assist the body in converting protein, fat, and carbohydrate into energy and making it accessible to the body. Vitamins C and E are antioxidants that also serve as cofactors for enzymes. Vitamin C has a variety of functions, including radical scavenging, collagen production, iron absorption, wound healing, and anti-inflammatory effects. Greater yam tubers have a greater concentration of vitamins than other tuber crops (USDA ARS, 2015). The most abundant nutrient in yam tubers is Vitamin C (Udensi et al., 2008).

Minerals

Dietary minerals are necessary for human nutrition and play an important part in the body's metabolic processes (Polycarp et al., 2012). Calcium is a necessary mineral that aids in blood coagulation and the integrity of intracellular cementing elements (Polycarp et al., 2012). Iron is required for the synthesis of blood haemoglobin and aids in the transfer of oxygen throughout the body. Myocardial illness, gastrointestinal infection, nasal bleeding, and other conditions are caused by a lack of iron in the body (Polycarp et al., 2012). Zinc is an important mineral that aids in the growth of the brain and bone, as well as wound healing (Padhan et al., 2018). It also aids in glucose, protein, vitamin A, and nucleic acid biosynthesis metabolic processes (Padhan et al., 2018). Potassium deficiency in the body enhances iron usage, which is excellent for hypertension management (Padhan and Panda, 2020). Potassium is good for diuretics who are trying to lower their blood pressure (Padhan and Panda, 2020)The greater yam tubers are high in minerals, with potassium being the most prevalent mineral found in the tubers (Baah et al., 2009; Polycarp et al., 2012).

Physico-functional properties

For bioprospecting of food ingredients, physico-functional properties such as water absorption capacity (WAC), foam capacity (FC), paste clarity (PC), water solubility index (WSI), and iodine affinity to starch (IAS), bulk density, and gelatinization temperature are important parameters in the food industry (Ohene Afoakwa and Simpson Budu, 2012). Starch and the ratio of amylose to amylopectin are two elements that determine the physico-functional characteristics (Sanful et al., 2013). Many researchers have researched many physico-functional properties in the yam tuber and have concluded that its flour may be used to make food products (Ojinnaka et al., 2017; Sanful et al., 2013). The quantity of water absorbed by flour to make dough of the desired consistency is known as water absorption capacity (WAC) (Chandra et al., 2015). The consequences of the flour's interactions with water and oil are reflected in the taste and texture of dishes. The flour with a high WAC is acceptable for use in the formulation of several culinary and bakery items that need viscosity (Chandra et al., 2015). The amylose leaching from starch granules is linked to the water solubility index (WSI) (Padhan & Panda, 2020). The texture, consistency, and look of food products are all improved by foam (Chandra et al., 2015). The foam capacity (FC) reveals how much interfacial area the protein in the flour creates (Chandra et al., 2015). The foam capacity and foam stability are inversely proportional (Chandra et al., 2015). Paste clarity (PC) is a desired quality that affects the food's brightness and turbidity (Mweta et al., 2008). Greater yam tuber flours, they said, have a lot of promise as a food component in the food sector. But, wild species such as *D. hamiltonii, D. pubera, and D. oppositifolia* showed greater WAC, FC, PC, WSI, and IAS values than farmed species *D. alata*, according to some researchers (Padhan and Panda, 2020). Hence, enhancement of these factors is to be done to this species to make it more usable in the commercialized food sector.

Anti-nutritional factors

Anti-nutritional factors are chemical compounds produced naturally by regular metabolism that impair

the body's nutrient consumption (Bhandari and Kawabata, 2004). Anti-nutritional factors lower the nutritive value of food by reducing the bioavailability of dietary components such as protein, minerals, and vitamins (Padhan et al., 2018). The acrid tubers of yam species contain anti-nutritional substances that cause skin irritation and inflammation of the buccal cavity and throat after ingestion (Kumar et al., 2017). Anti-nutritional elements in yams include phenols, alkaloid, oxalate, phytate, tannin, saponin, amylase inhibitors, and trypsin inhibitors, which are responsible for toxicity and bitterness (Poornima and Ravishankar, 2009).

Yam tubers have a higher concentration of alpha amylase inhibitor than other commercial tuber crops (Padhan et al., 2020; Polycarp et al., 2012). The amylase inhibitor creates a compound with pancreatic amylase enzyme in a 1:1 ratio and attaches to a place other than the active site, inactivating the enzyme's catalytic power via conformational changes (Jamil et al., 2000). Protease inhibitors are a group of proteins that suppress proteolytic enzymes. Trypsin inhibitor is one of them. The concentration of trypsin inhibitors in wild yam tubers has been observed to be higher than in farmed species (Bhandari and Kawabata, 2006; Padhan et al., 2020). Alkaloids and their derivatives exhibit a variety of pharmacological effects, including analgesic, antispasmodic, and antibacterial activities (Polycarp et al., 2012). The alkaloid content of wild yam species is said to be higher than that of farmed yam species (Polycarp et al., 2012). The antioxidant activity of flavonoids found in yam species has been proven to scavenge free radicals (Bhandari and Kawabata, 2004). Tannins are the compounds that give foods and beverages their astringent flavour. Plants with high tannin content have been used to cure disorders including leucorrhoea, rhinorrhoea, wound healing, and diarrhoea (Eleazu et al., 2013). The presence of tannins in yam species causes them to be bitter (Padhan et al., 2020).

The principal physiologically active elements of yams have been found to be steroidal saponins (Avula et al., 2012). There are 20 distinct forms of steroidal saponins in *Dioscorea* species, each with different pharmacological effects (Avula et al., 2012). Saponins from yam species have been utilised to make steroid medicines in the past (Kumar et al., 2017). Phenolic chemicals act as a growth inhibitor in plants. They are generally found in conjunction with glucosyl residues in plant tissue. Phenols are classified as anti-nutrients because they reduce protein, carbohydrate, and mineral digestibility and hence render them insoluble (Padhan et al., 2018). They also stop digestive enzymes like amylase, trypsin, and chymotrypsin from working, causing injury to the mucosa of the digestive system (Bhandari and Kawabata, 2004). When the tuber flesh is exposed to the air, the phenols from the larger yam tubers are the main source of browning (Bhandari and Kawabata, 2004). The presence of phenol in higher yam helps to the antioxidant capacity, according to researchers (Cornago et al., 2011; Niu et al., 2010). Oxalate is found in plants in the form of calcium oxalate and is extensively spread. Oxalic acid forms oxalate salts when it reacts strongly with dietary elements such calcium, magnesium, sodium, and potassium (Padhan et al., 2018). Calcium oxalate crystals occur when insoluble calcium oxalate salts precipitate in the kidney and urinary system, causing kidney stones (Padhan et al., 2018). Increased oxalate levels in diet induce nutritional deficiencies as well as severe throat discomfort. The presence of calcium oxalate crystals (raphides) in increased yam mucilage promotes skin and mucous membrane irritation (Otegbayo et al., 2018). Wild yam species have been shown to have a higher concentration of oxalates, which cause skin irritation and throat discomfort (Bhandari and Kawabata, 2004; Polycarp et al., 2012).

Bioactive components

The bioactive components are secondary metabolites derived from plants that are employed in insect and pest defence mechanisms. Phenols, polyphenols, alkaloids, polypeptides, steroids, terpenoids, and essential oils are bioactive components with a variety of pharmacological actions (Alamu et al., 2014). Bioactive substances such as phenols, alkaloids, tannins, flavonoids, saponins, glycoside steroids, anthraquinones, and others are known to be abundant in *Dioscorea* species (Price et al., 2017).

Conclusion

Greater yam *(D. alata)* is the world's most popular yam after the *D. rotundata/cayenensis* complex and appears to have held its place. Although traditional methods of production (especially in Africa) are more costly in manpower than for other yams, the introduction of complete field mechanisation, which is now a reality, should reduce production costs and make this crop more competitive as a tropical carbohydrate food and also enable it to maintain or improve its position on the export market. No figures are available for the production of *D. alata* separately from other yams. There has been a small export trade in *D. alata* from some of the Caribbean islands to the UK since the early 1960s. In 1968, approximately 1000 t of tubers of *D. alata* were exported from Barbados, but the occurrence of chilling injury at the receiving point, and the incidence of internal black spot (virus), reduced the trade almost to zero. However, the recent production of virus-free yams has allowed the trade to re-start bringing on the new era for yam.

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Standardization of an efficient DNA isolation protocol in tannia [*Xanthosoma sagittifolium*(L.) Schott]

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Abstract

Genetic variability is very much limited in tannia (*Xanthosoma sagittifolium*(L.) Schott).Morphological variation is limited to colour variation in leaf, petioleand tuber flesh colour in this crop. Tuber shape and size also shows variation. ICAR-CTCRI being the National Repository for tuber crops is maintaining the germplasm collection of edible aroids including tannia. In order to assess the genetic diversity existing in this introduced crop, different DNA isolation protocols were tested in four tannia accessions to identity the best method. CTAB method standardised by Sharma et al., (2008) for taro, DNeasy kit method (Qiagen) and modified Dellaporta method were tried. It was observed that the quality of DNA was good in the modified Dellaporta method with DNA quality ranging from 1.98 (Xa-6) to 2.23 (Xa-67). Good quantity was obtained ranging from 830 ng/μl (ACIX-2) to 1968 ng/μl (Xa-71). The extracted DNA was amenable to ISSR markers. Hence, for all further molecular studies in tannia, the modified Dellaporta method was adopted.

Keywords: Tannia, *Xanthosoma sagittifolium*, DNA isolation, DNA quantity, DNA quality, Nanodrop spectrophotometer

Introduction

Tannia (*Xanthosoma sagittifolium*) is a staple root crop that belongs to the family Araceae. It is a native of tropical, central and South America as well as the Caribbean which is cultivated for its starchy edible tubers. It is a cultivated crop in India, grown for its edible and starchy tubers as well as tender edible leaves. The plant resembles the common taro (*Colocasia esculenta*) in its morphological characters, especially the tubers and the leaves to some extent. In taro, the petiole attachment is in leaf lamina (peltate), whereas in tannia, it is at the base of the lamina (sagittate). Because of its resemblance to taro, which is called cocoyam in some locations, tannia became known as the new cocoyam. (Rubatzky and Yamaguchi, 1997).

The centre of origin for tannia is reported to be tropical America but it is now cultivated throughout Africa, Asia and the Pacific territories. Movement of this crop from its tropical north-eastern south American origin was relatively recent. The crop was taken to Africa during the slave trading era and from there it was rapidly adopted to the other regions and now ranks behind cassava and yams in importance. This crop comes up well under tropical conditions with a mean temperature of above 21°C. In India, tannia is cultivated in the north-eastern states and southern parts of the country (Das et al., 2016). The crop is also amenable to organic manures (Suja et al., 2009).

A herbaceous perennial, *X. sagittifolium* has a corm or main underground stem in the form of a rhizome from

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which swollen secondary shoots, or cormels sprout. Several large leaves also sprout from the main stem, which are sagittate and erect with long, ribbed petioles; inflorescences sprout between the leaves in a spadix, with a white 12 to 15 cm spathe which closes at its base in the form of a spherical chamber and opens at the top into a concave lamina; the spadix is cylindrical, slightly longer than the spathe, with female flowers on the lower portion, male flowers on the upper portion and sterile flowers in the middle portion. The spadices are rarely fertile and produce few viable seeds.

The corm, cormels and leaves are the main economically important parts of the plant. The main corm (mother corm) is acrid and hence only the daughter cormels (side corms) are consumed. The cormels which are consumed as food contains 17-26% carbohydrate, 1.3-3.7% protein and 65-77% water and have nutritional value comparable to potato (Onwueme and Charles, 1994; Agueguia, 2000). The carbohydrate part is mostly composed of starch which is relatively large grains with an average diameter of 17-20 μm (Onwueme, 1978). This makes tannia starch, less readily digested than that of taro. Like taro, the corms of tannia contains raphides and should not be fed to human or livestock without cooking. *X. sagittifolium*is used as a medicinal species both as food and medicine to prevent and treat bone diseases, such as osteoporosis, in traditional Brazilian medicine (de Oliveiraet al., 2012).

Mostly owing to its introduced nature, genetic variation is very limited in this crop cultivated in India. Morphological variation is seen in the colour of leaf, petiole and tuber flesh. Tuber shape and size also shows variation. Majority of the collections present in the National Repository maintained at ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI) have green colour petiole whereas, a few have purple colour too. According to Sepúlveda-Nieto et al. (2017), there are some distinctive morphological markers for identification of tannia such as: leaves with subcoriaceous textures, basal insertion of the petiole, green pseudo-stem in the basal portion with exudate being white and the presence of two collector veins. However, due to their similar phenotypic characteristics, especially in the form and colour of the leaves and petioles, *X. sagittifolium* (tannia) and *C. esculenta* (taro) are popularly mistaken for one another (Onwueme, 1999; Onyeka, 2014). In such cases, molecular studies have been useful in identifying the genetic differences between species which closely resemble one another. Within the realm of molecular markers, Inter-Simple Sequence Repeat (ISSR) markers have proven to be highly effective in the discrimination of diverse taxa. To initiate molecular studies in tannia, different DNA isolation protocols were tested using four tannia accessions to assess the suitability of the method in obtaining good quality DNA for studying the genetic diversity existing in this introduced crop.

Materials and Methods

Source of sample

The study was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram. Four accessions of *Xanthosoma sagittifolium*(tannia) conserved and maintained in the ICAR-CTCRI National repository were selected for the present study (Table 1). The plants were raised in the field and young leaves from 5–6 month-old plants were used for DNA isolation.

Sl. No.	Accession name	Species name	Place of collection
1	Xa 6	*X. sagittifolium*	Unknown
2	Xa 71	*X. sagittifolium*	Unknown
3	ACIX 2	*X. sagittifolium*	Kerala
4	Xa 67	*X. sagittifolium*	Kerala

DNA extraction methods

Three DNA isolation methods viz., CTAB method standardised by Sharma et al., (2008) for taro, DNeasy kit method (Qiagen) and modified Dellaporta method were tried. Modified CTAB method standardised for taro (Sharma et al., 2008). The young leaves from 5-6 months old tannia plants were collected and weighed 1.5 g avoiding the mid rib regions and veins and crushed in liquid nitrogen. The extraction buffer consisting of 100 mM tris-HCl (pH 8), 20 mM; EDTA (pH 8), 2 M NaCl, 2% CTAB (w/v), 2% PVP (Mw 40,000), 2%b-mercaptoethanol (v/v) was prepared. Freshly prepared buffer was preheated at 65°C, added to the above powder and gently homogenized. The homogenate was transferred to 2 ml microfuge tubes, labelled and kept in a water bath at 65°C for 45 min with intermittent shaking. The samples were then centrifuged at 12,000 rpm for 10 min.The supernatant was added with equal volume of 24:1 (v/v) chloroform/isoamyl alcohol andagain centrifuged at 12,000 rpm for 10 min. The supernatant was taken and 5 μl of RNase A (10 mg ml^{-1}) was added and mixed gently by inversion. The samples were then incubated in a dry bath at 37°C for 45 min and added with equal volume of chloroform: isoamyl alcohol (24:1) and centrifuged at 12,000 rpm for 10 min. The upper aqueous layer was taken and treated with 2/3 volume of iso-propanol. The precipitated DNA was pelletized by centrifuging at 10,000 rpm for 15 min. and then washed with 70% ethanol by spinning at 10,000 rpm for 5 min. The DNA pellets were air dried to remove any traces of ethanol followed by suspension in 50-100μl of 1X TE buffer. The DNA suspension was stored at -20°C for further use.

DNeasy® plant mini kit (Qiagen) method

The leaf samples were disrupted using a mortar and pestle. Then the protocol given in the kit was followed.

Added 400 µl buffer AP 1 and 4 µ RNase A, vortexed and incubated for 10 min. at 65^{0}C. The tubes were inverted 2-3 times during incubation and 130 µl buffer P 3 was added, mixed and incubated for 5 min. on ice. The lysate was centrifuged for 5 min. at 14,000 rpm and pipetted into a Q I A shredder spin column placed in a 2 ml collection tube and centrifuged for 2 min. at 14,000 rpm. The flow-through was transferred into a new tube without disturbing the pellet, if present. Then 1.5 volumes of buffer AW 1was added, and mixed by pipetting. The mixture (650 µl)was transferred into a DNeasy mini spin column placed in a 2ml collection tube and centrifuged for 1min. at 8000 rpm., the flow-through was discarded. This step was repeated,and the spin column was placed into a new 2 ml collection tube. Then, 500 µl buffer AW 2 was added and centrifuged for 1min. at 8000 rpm. The flow-through was discarded. Then added another 500 µl of buffer AW 2, centrifuged for 2 min at 14,000 rpm. and transferred the spin column to a 1.5 ml or 2 ml micro centrifuge tube. Added another 100 µl buffer AE for elution. The mixture was incubated for 15 minutes at room temperature. Then centrifuged for 1 min at >600 × g. This step was repeated, and the eluted DNA was stored at -20°C.

Modified Dellaporta method (Dellaporta et al., 1983)

One g of leaf bits were taken from the young tender leaves and were transferred into a pre-chilled mortar and pestle, frozen already using liquid nitrogen and ground to a fine powder. The powdered samples were mixed with 15 ml of extraction buffer containing 100 mM tris-HCl (pH 8), 20 mM; EDTA (pH 8), 2 M NaCl, 2% PVP (Mw 40,000), 0.2% ß-mercaptoethanol (v/v) was added and kept at 4°C. To this mixture, 1ml of 20% SDS was added, thoroughly mixed, and incubated at 65°C for 1 h in a water bath. Five ml of 5M potassium acetate was then added and kept on ice for 20 min. and centrifuged at 12,000 rpm for 20 min. and the clear aqueous phase was transferred to a new sterile tube. Equal volume of ice-cold isopropanol was added and mixed gently by inversion and then kept in the freezer until DNA was precipitated out. Centrifugation was performed at 12,000 rpm for 10 min., the pellet obtained was dissolved in 500 µl sterile double distilled water and transferred to a microfuge tube. Five µl of RNase A (10 mg ml^{-1}) was added and incubated at 37°C for one h. Five hundred µl of 24:1 (v/v) chloroform:isoamylalcoholwas then added, mixed well and centrifuged at 12,000 rpm for 15 min. The supernatant was collected, added with two volumes ice cold absolute ethanol and 1/10volume 3M sodium acetate and kept overnight incubation or one h in -20°C. It was centrifuged at 12,000 rpm for 10 min. and the supernatant was discarded. To the pellet, 500 µl of 70% ethanol was added to wash the DNA. Then the alcohol was discarded, and the DNA was air dried fully. The DNA was then dissolved in 500 µl of TE buffer and stored the sample at -20°C.

Qualitative and quantitative analysis of extracted DNA

The DNA yield was measured by using a nanodrop spectrophotometer (Denovix DS-11+) at 260 nm. The DNA purity was determined by calculating the absorbance ratio A260/280. For quality and yield assessments, electrophoresis was done of all DNA samples in 1% agarose gel, stained with ethidium bromide and bands were observed in gel documentation system (Alpha Innotech).

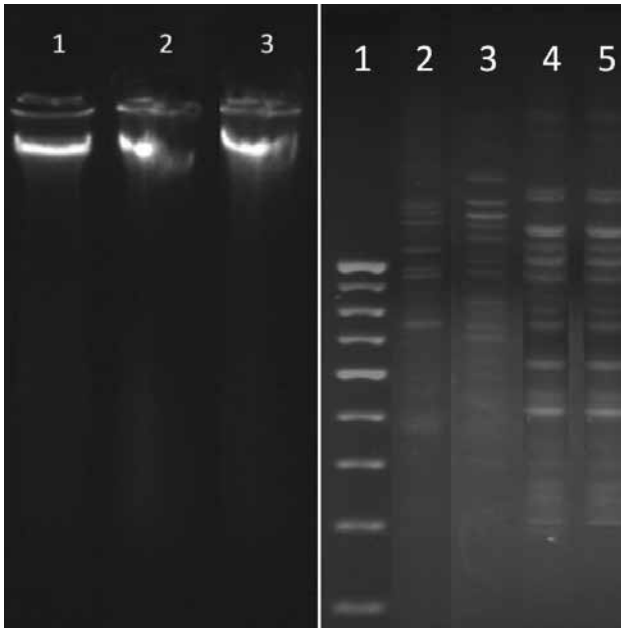
Inter Simple Sequence Repeat (ISSR) study

The PCR amplification reaction was carried out with nine ISSR markers in a 20 µl reaction volume containing 10 mM Tris-HCl, pH 8.3, 20 mM $MgCl_2$, 1 mM dNTP mix, 0.2 µM of each primer, 1 U of Taq DNA polymerase, and 10 ng of template DNA. ISSR-PCR was performed in a thermal cycler (PTC-100tm MJ Research Inc., USA) for 35 cycles consisting of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, and extension at 72°C for 2 min. The final extension was carried out at the same temperature for 5 min. The amplified product was checked in 2% agarose gel electrophoresis and bands were observed in gel documentation system (Alpha Innotech).

Results and Discussion

Fresh young leaves were used for the isolation of goodquality DNA as mature leaves contain higher quantities of polyphenols and polysaccharides (Porebski et al., 1997), making it difficult to isolate DNA of good quality. The isolation of pure, intact, and high-quality DNA is a very important step for any molecular studies. DNA isolation methods need to be adjusted to each plant species and even to each plant tissue because of the presence of metabolites in them, unlike animals and microbes (Sangwan etal., 1998). In the present study, three different DNA isolation protocols were tested. The quality and quantity of DNA was tested using a Nanodrop spectrophotometer and agarose gel electrophoresis (1%), respectively to ensure the use of good quality of DNA for molecular marker studies. Among all the tested protocols, the modified Dellaporta method yielded good results. The modified CTAB extraction and Qiagenkit method did not show promising results for the four tannia accessions as evidenced by the sheared band in the agarose gel (Fig. 1a).

The quantity of DNA present in each sample as determined by the 260/280 value using a nanodrop spectrophotometer are shown in Table 2. Good quality DNA was obtained from the four accessions studied and it ranged between 1.98 (Xa-6) to 2.23 (Xa-67). DNA readings of 1.8 - 2.0 are considered very good



(a) (b)

Fig. 1. (a) DNA isolated using the different protocols in tannia accession Xa-67 (Lane1: modified Dellaporta method; Lane2: Sharma et al., 2008; Lane3: DNeasy Qiagen kit method), (b) ISSR pattern of tannia accessions (Lane1: 100 bp ladder; Lane2 to Lane5: tannia accessions)

for molecular studies. Washing with Chloroform: isoamylalcohol treatment ensures removal of chlorophyll, pigments, and dyes (Sahu et al., 2012) thus improving the quality of DNA. The above method yielded good quantity ranging from 830 ng/μl (ACIX-2) to 1968 ng/μl (Xa-71). In the modified Dellaporta method, the pre-cooling of the mortar and pestle, use of ice cold isopropanol and refrigeration steps had a positive effect on the DNA extracted. Here, SDS was used instead of CTAB. Addition of potassium acetate served the purpose of facilitating the removal of a significant portion of the proteins and polysaccharides within the sample, forming a complex with the insoluble potassium dodecyl sulphate precipitate (Dellaporta et al., 1983).

Table 2. Quantity and quality of DNA isolated using the modified Dellaporta method as assessed using nanodrop spectrophotometer

Sl. No.	Accession name	OD 260/280	Concentration (ng/μl)
1	Xa 6	1.98	1219
2	Xa 71	2.06	1968
3	ACIX 2	2.21	830
4	Xa 67	2.23	976

It was noted that the extracted DNA was amenable to the ISSR markers tested. Clear banding patterns were observed in the ISSR study (Fig.1b) and hence, this modified Dellaporta method was employed for tannia.

Conclusion

The study's findings led to the conclusion that for obtaining high-quality DNA, the modified Dellaporta method proved effective and was also suitable for ISSR marker analysis, resulting in distinct and consistent bands. Therefore, this method is recommended for the isolation of genomic DNA from tannia.

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Effect of various soil amendments on soil pH and exchangeable Ca at different depths in the identification of a suitable liming material for tannia (*Xanthosoma sagittifolium* L. Schott) in an Ultisol, Kerala, India

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Abstract

Tannia (*Xanthosoma sagittifolium* L. Schott) is an important tropical tuber crop coming under the group of aroids. It is usually grown as intercrop in plantations and fetches good remuneration. When this crop is grown in the acid laterite soils (Ultisols), widespread occurrence of Mg deficiency was very common resulting in the complete devastation of the crop. The reason attributed was the acidity due to excess Al^{3+}ions in the subsoil layers which causes the decay of roots and hence adversely affecting the absorption of water and nutrients. This crop being an indicator plant for Mg deficiency, the symptom is manifested as interveinal chlorosis coupled with drying of the whole plant. The present study was undertaken to screen a better soil amendment to tackle the problem of subsoil acidity induced multi nutrient disorder in tannia. The present study was done using five liming materials *viz.,* calcite, dolomite, gypsum, calcium silicate and calcium oxalate at quantities ranging from 50-1000g both under controlled condition in lysimeter and in field. After application of treatments, soil samples drawn continuously for 6-7 months from four different depths *viz.,* 0-15, 15-30, 30-45 and 45-60 cm were analysed for pH, CEC and exchangeable Ca. The data generated were statistically analysed for the independent and interaction effect of treatments (liming materials, rate of application, depth of sampling and interval after application) and results on the mean effect, changes over initial and at the end of 6-7 months of application with respect pH and exchangeable Ca for the above treatments were described. The results from both situations revealed that, calcite, dolomite and gypsum were equally effective. Among the three, dolomite being a source of both Ca and Mg, even at lower quantities as 250 g was found better in enhancing both pH (1.54 units over initial) and exchangeable Ca (1.559 cmol (+) kg $soil^{-1}$ over initial) to the maximum extent especially at lower depths up to 60 cm to a period of 4-5 months after application.

Keywords: Tannia, Subsoil acidity, Mg deficiency, Liming materials, Exchangeable Ca

Introduction

Among the tropical tuber crops, aroids *viz.,* elephant foot yam, taro and tannia are valued as nutritious as well as therapeutically valuable tuberous vegetables grown mainly as intercrops in coconut as well as in banana and rubber plantations of Kerala. Since coconut is a major crop grown both on plantation scale and component crop in the homesteads of Kerala, raising intercrops like aroids is a common practice by farmers. Among the aroids, tannia known as new cocoyam (*Xanthosoma sagittifolium* L.Schott) is the most remunerative as its cormelsfetches good price in local markets and is having good export potential.

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Tannia is cultivated for edible tubers (corms and cormels) and young leaves. The tubers and young leaves are highly nutritious having substantial quantities of protein, vitamins and minerals in addition to starch/ energy. Compared to taro, tannia is preferred by people due to the absence of itching and lack of too much of mucilage in the tubers, good cooking quality as well as better taste. Since the starch granules in tannia tubers are relatively large, they are mainly used for the production of industrial starch. In some places, flesh scrapings of corm and cormels are pulped and used as anti-coagulants, anti-tetanus and anti-venom agents against tarantula, scorpion and even snake bites.

When tannia was grown in acid laterite soils (Ultisols), the crop will be having luxuriant vegetative growth up to 3-4 month after planting (MAP) if other growth conditions like soil moisture, sunlight and relative humidity are adequate. After that, the crop starts showing clear and distinguishable symptoms in the foliage characteristic of Mg deficiency in the form of interveinal chlorosis of older leaves followed by cupping inward and drying up of the tip and margins of the entire leaf. In severe cases, the entire foliage will dry up resulting in complete devastation of the crop without any tuber formation and development. In most of the situations, this is accompanied by expression of K deficiency symptoms as characteristic marginal drying and necrosis of lower leaves extending to the leaf tip resulting in complete drying and falling off and Ca deficiency in the form of crinkling of younger leaves with reduction in leaf size and difficulty of leaves to emerge.

The above observations were substantiated through the analysis of soil and leaf samples which in turn indicated that, the soils (Ultisols) where the crop was grown was acidic to very strongly acidic with a pH range of 4.5-5.0 and quite deficient in Mg, Ca and K. This very low pH indicates the excess availability of Al^{3+} ions and toxic levels of Al do inhibit the root growth of the plant inducing the non-availability of secondary nutrients especially Ca and Mg. This may also cause water stress resulting in the occurrence of nutritional disorders due to Mg, Ca, and K in Ultisols or laterite soils even after the grand growth period is crossed. Moreover, as Mg, K and Ca are prone to leaching under tropical conditions, they will become unavailable to the plant especially during the critical vegetative growth and at the initiation of tuber bulking. As the amount of basic cations especially Mg available in the soil during the critical growth stages may not be sufficient to meet the crop demand (Susan John et al., 2006). As Tannia is an indicator plant for Mg deficiency having lower Mg and excess Ca contents in Mg deficient tannia leaves, the effect of subsoil acidity is manifested as Mg deficiency (Cable, 1995). Nair et al., (2018) already reported the prevalence of strongly acid condition in the surface and subsurface soils of Kerala.

After recognizing the problem in tannia as subsoil acidity induced multi nutrient disorder involving nutrients like K, Ca, and Mg, in order to screen a suitable soil ameliorant for this crop to manage subsoil acidity, the present study was carried out with liming materials like lime, dolomite, gypsum, calcium silicate and calcium oxalate. As there are many reports (Anikwe et al., 2016; Thomas and Hargrove, 1984; Shainberg et al., 1989; Rashid and James, 1980) indicating the usefulness of different liming materials in correcting surface and subsurface acidic conditions, these five liming materials containing Ca and Mg with different quantities at different depth of application were tested in the present study.

Materials and Methods

This basic study was undertaken based on the results of experiments conducted to rectify the soil acidity related nutritional disorders associated with the manifestation of the foliar symptom and consequent tuber initiation and development problems. The primary objective was to identify a suitable liming material to correct the subsoil acidity, so that, the crop can be saved from the existing nutritional disorder.

Here, the experiments were conducted both under field as well as under controlled condition in lysimeter tanks of 1 m^3 volume using five different liming materials *viz.,* calcite, dolomite, gypsum, calcium silicate and calcium oxalate at different rates containing calcium oxide (CaO) to the tune of 56, 33.2, 32.2, 32.6 and 43.8% respectively. The liming materials used, their rate of application as well as the depth of sampling both under lysimeter and field condition are given in Table 1.

These soil samples were analysed for pH (pH meter, Systronics), CEC and exchangeable Ca (Page et al., 1982). In the case of lysimeter studies, pH, exchangeable Ca and CEC was determined initially from the surface (0-15 cm) soil. After application of the above treatments in lysimeter, pH was measured for a period of 7 months continuously from the above four depths, Ca for a period of 6 months continuously from the above four depths and CEC only initial and after 5 months from the surface soil.

In the field situation, the initial samples were analysed for pH, and exchangeable Ca from the surface soil and pH and Ca were analysed from the above four depths for a period of 6 months continuously from the first month of application onwards. Statistical analysis of the data was carried out for independent and interaction effect of treatments *viz.,* liming materials (calcite, dolomite, gypsum, calcium silicate, calcium oxalate) soil depth (0-15, 15-30, 30-45, 45-60 cm), quantity (50,100,150 and 200g in the case of calcium silicate and calcium oxalate and 250,500,750 and 1000g in the case of calcite, dolomite, and gypsum), intervals (initial, 1,2,3,4,5,6,7

Table 1. Liming materials used, rate and depth of soil sampling

Method	Liming material	Quantity/tank	Depth
Lysimeter	Calcite	500g	0-15,15-30,30-45and 45-60cm
	Dolomite	500g	0-15,15-30,30-45and 45-60cm
	Gypsum	500g	0-15,15-30,30-45and 45-60cm
	Calcium silicate	100g	0-15,15-30,30-45and 45-60cm
	Calcium oxalate	100g	0-15,15-30,30-45and 45-60cm
Field	Calcite	@ 250,500,750 1000g m^{-3}	0-15,15-30,30-45and 45-60cm
	Dolomite	@ 250, 500,750 1000 m^{-3}	0-15,15-30,30-45and 45-60cm
	Gypsum	@ 250, 500,750 1000g m^{-3}	0-15,15-30,30-45and 45-60cm
	Calcium silicate	@ 50, 100,150, 200g m^{-3}	0-15,15-30,30-45and 45-60cm
	Calcium oxalate	@ 50, 100,150 200g m^{-3}	0-15,15-30,30-45and 45-60cm

months after application (MAA) in the case of pH, initial and one month interval continuously up to 6 MAA in the case of exchangeable Ca and initial and 5 MAA in the case of CEC using Genstat (2010).

The basic soil parameters of the soil of the experimental plot under field condition is given below: Soil type: sandy clay loam, pH: 4.3-4.9, Organic Carbon: 0.53 -0.70 %, Available N: 175 kg ha^{-1}, Available P: 68 kg ha^{-1}, Exchangeable K: 193 kg ha^{-1}, Exchangeable Ca: 0.74 cmol (+) kg^{-1} soil, Exchangeable Mg: 0.56 cmol (+) kg^{-1} soil, Available S: 7.3-12.4 ppm, Available Fe: 21 ppm, Available Cu: 0.34 ppm, Available Mn : 8.3 ppm, Available Zn: 2.6 ppm, Available B: 0.68 ppm

Results and Discussion

The results of the experiments carried out both in lysimeter and under field condition with the above liming materials in the screening of the best soil amendment for tannia in the Ultisols of Kerala are detailed below:

1. Lysimeter

The initial soil samples analysed from the surface for the five treatments as shown in Table 1 as well as control indicated the pH as near neutral with a mean value of 6.89 and ranged from 6.37 to 7.31. Similarly, the exchangeable Ca indicated a mean value of 5.14 cmol (+) kg^{-1} soil with ranges as 4.31 to 5.96 cmol (+) kg^{-1} soil and CEC in the range of 12.06-16.51 cmol(+) kg $soil^{-1}$ with a mean value of 13.34 cmol(+) kg $soil^{-1}$

Table 2. Initial pH, CEC and exchangeable Ca of the soil under lysimeter experiment

Liming material	Lysimeter (0-15 cm)		
	pH	Ca	CEC
		(cmol (+) kg^{-1} soil)	
Calcite	6.37	4.31	12.06
Dolomite	6.88	4.53	13.03
Gypsum	6.73	5.72	13.25
Calcium Silicate	7.07	4.99	10.86
Calcium oxalate	6.98	5.33	16.51
Absolute control	7.31	5.96	14.34
Mean	6.89	5.14	13.34

Effect of liming materials

Changes in pH

The mean pH determined over a period of 7 months indicated the effect of the liming materials as same on pH except for calcium silicate which has resulted in a significantly low pH compared to the other four liming materials. This in turn corroborates to the findings of Thomas and Hargrove (1984) revealing the effect as complete absorption and retention of the added Ca through the replacement of exchangeable Al^{3+} ions.The overall improvement in pH over initial was seen with calcite and gypsum only, to the tune of 3.44 and 1.29%. This increase might have been due to the movement of labile Ca down the soil decreasing the Al in the subsoil layers as well as due to the formation and precipitation of $AlSO_4$ as reported by Pavan et al., (1984). As regards to the pH after 7 months of its application, it was seen that, the change in pH was statistically similar for all liming materials except for calcium silicate which resulted in a

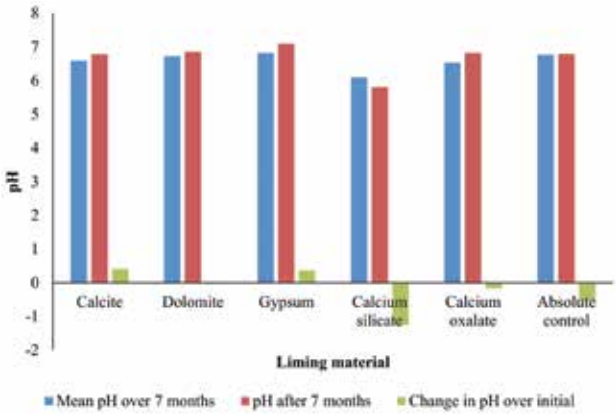

Fig.1. Changes in pH under different liming materials over a period of 7 months

significantly low pH. The change in pH over the initial showed increase in the case of calcite and gypsum. The change over initial as well as the % change is given in Fig.1.

Changes in exchangeable Ca

The overall mean soil exchangeable Ca over the 6 months indicated significant effect of liming materials on soil exchangeable Ca with gypsum resulted in significantly higher Ca may be the effect of self-liming as reported by Reeve and Sumner (1972). Calcium silicate resulted in significantly low available soil Ca on par with that of calcium oxalate, calcite and dolomite. At the end of 6 months of application, no significant effect of liming materials was seen on soil exchangeable Ca. However, gypsum followed by dolomite, calcite and calcium oxalate resulted in comparatively high soil Ca. Over the initial Ca, at 6 MAA, the Ca content was seen depleted with all liming materials in the order as control, the highest followed by calcium silicate, calcium oxalate, gypsum and then dolomite and calcite (Table 4). The comparatively low Ca associated with calcium silicate might have been the effect of restricted Ca movement with calcium silicate as reported by Mahilum et al., (1970).

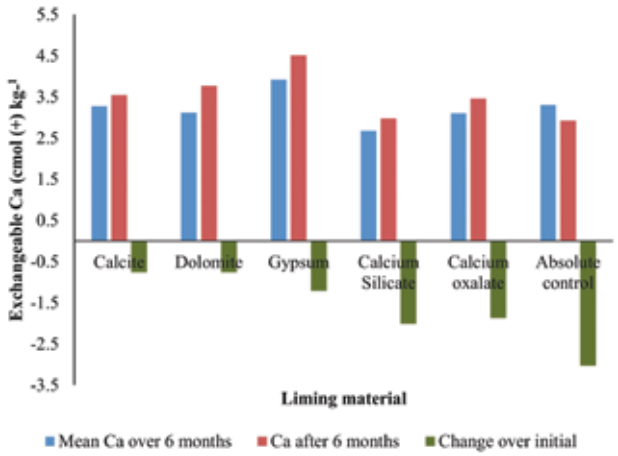

Fig. 2. Changes in exchangeable Ca under different liming materials over a period of 6 months

Changes in soil CEC

After 5 months, the CEC estimated showed a drastic reduction compared to initial in the case of all liming materials viz., calcite, dolomite, gypsum, calcium silicate, calcium oxalate and absolute control to the tune of 1.91, 20.80, 43.09, 14, 40.94 and 24.27% respectively. Edmeades (1982) in a range of New Zealand soils reported that, there will not be much retention of cations other than calcium in the exchange sites due to liming and hence cannot expect an increase in CEC.

Effect of depth

In the case of soil pH, over these 7 months, the pH was significantly higher in 15-30 cm and was on par with 0-15 cm depth. The soil pH was significantly lowest at 45-60 cm depth. The exchangeable Ca at 0-15 cm was significantly the highest and the Ca content at 15-30 cm depth was on par with that at 30-45 cm. The soil depth of 45-60 cm significantly registered the least soil exchangeable Ca. High pH and exchangeable Ca noted in the surface 0-30 cm depth was apparently more as the liming materials reacted with the surface soil and less moved to lower layers during the seven months of incubation as seen in an experiment in a tropical soil profile by Rashid and James(1980).

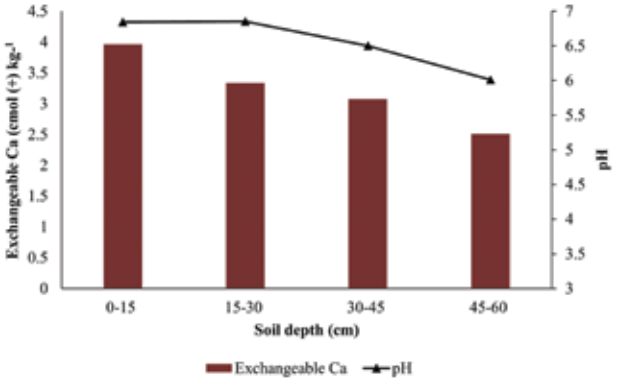

Fig. 3. Changes in pH and exchangeable Ca under different liming materials over period of time at different depths

Quantity of liming materials

In the lysimeter tanks, we used two quantities of the liming materials *viz*., calcite, dolomite and gypsum @ 500g each and calcium silicate and calcium oxalate @ 100g each. Over these months, the overall pH under these quantities differed significantly with 500g resulted in significantly the highest soil pH. Similar was the effect on soil exchangeable Ca too. Qaswar et al., (2020) reported high pH values, exchangeable cations including Ca and consequently low exchangeable Al and exchange acidity with high lime rates.

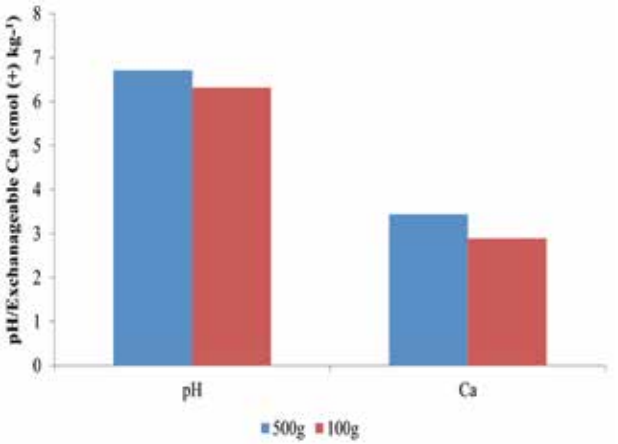

Fig. 4. Changes in pH and exchangeable Ca under two rates of different liming materials over a period of 7 months at different depths

Months after application of liming materials

After application of the different liming materials, samples were drawn and analysed for pH up to 7months and exchangeable Ca up to 6 months. Statistical analysis of the data for the effect of intervals alone and lime interval interaction on both pH and exchangeable Ca indicated significant effect of intervals alone on both pH and Ca and the interaction was non-significant.

It was seen that, the pH after 4, 5 and 7 MAA was on par with that of the initial which in turn was highest among the different intervals. The pH recorded at 1 MAA was significantly lower and was on par with the pH at 2,3,4 and 6 MAA. In the case of exchangeable Ca at different intervals, the initial exchangeable Ca was significantly the highest. The Ca content at 5 and 6 MAA were significantly higher and were on par. During the other intervals, the Ca content was significantly lower and was on par. Similar findings of increase in soil pH and exchangeable cations were reported by da Costa et al., (2016) while taking observation at 48 and 60 months after application of liming materials in a tropical clayey Oxisol.

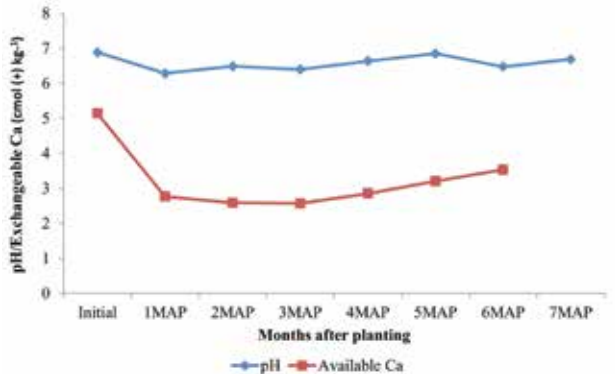

Fig. 5. pH and exchangeable Ca under two rates of different liming materials at different intervals at different depths

2. Field Condition

The effect of application of five different liming materials *viz.,*calcite, dolomite and gypsum @ 250, 500, 750 and 1000g and calcium silicate and calcium oxalate @ 50, 100, 150 and 200g at four different depths as 0-15,15-30,30-45 and 45-60 cm from application for a period of 6 months continuously is described below based on the statistical analysis of the data in Genstat (2010).

The initial soil pH and exchangeable Ca at the four different depths are given in Table 3.

Table 3. Initial soil pH and exchangeable Ca of the experimental site at four depths

Depth (cm)	pH	Exchangeable Ca (cmol (+) kg soil^{-1})
0-15	5.25	0.59
15-30	5.34	0.61
30-45	5.04	0.44
45-60	4.62	0.18
Mean	5.06	0.46

The depth wise analysis of the initial soil samples for pH and exchangeable Ca indicated the mean pH and Ca of the initial soil samples as 5.06 and 0.46 meq 100g^{-1} respectively. Both pH as well as Ca was found higher at 15-30 cm soil depth followed by 0-15, 30-45 and 45-60 cm.

Effect of liming materials

Table 4. Changes in pH under different liming materials over a period of 6 months

Liming material	Mean pH	pH after 6 months	Change in pH over initial	Percentage Change in pH over initial
Calcite	5.83	6.38	1.32	26.09
Dolomite	6.14	6.60	1.54	30.42
Gypsum	5.17	6.16	1.10	21.68
Calcium Silicate	5.35	5.57	0.51	10.02
Calcium oxalate	5.04	5.39	0.33	6.44
Absolute control	4.95	5.48	0.42	8.30
CD(0.05)	0.188	0.363		

The effect of liming materials on pH over these 6 months indicated, dolomite followed by calcite as the best as the pH under these treatments were significantly the highest, followed by calcium silicate which was on par with gypsum which in turn was on par with calcium oxalate. At the end of six months, it is seen that, the highest increase in pH was for dolomite followed by calcite, gypsum, calcium silicate and calcium oxalate resulted in significantly the lowest even less than absolute control (Table 4). Susan John et al., (2013) reported dolomite as the best liming material for Ultisols of Kerala. In the case of exchangeable Ca, dolomite resulted in significantly higher exchangeable Ca followed by calcite on par with gypsum followed by calcium silicate and calcium oxalate, significantly the least even less than absolute control. After 6 months of the application of these amendments, it is seen that, dolomite, followed by calcite and gypsum resulted in significantly higher exchangeable Ca and were on par and then calcium silicate (Table 5). Anikwe

et al., (2016) had similar observations with dolomite, lime and gypsum under cassava in an Ultisol of Nigeria. Calcium oxalate resulted in significantly the least on par with absolute control and the same trend was seen in the case of increase in exchangeable Ca over these six months. However, the increase in Ca noticed with all these amendments was substantial. The substantially low pH and exchangeable Ca associated with calcium oxalate can be attributed to the reports of Uren (2018) that, in acid soils, the microbiological transformation of calcium oxalate to Ca through the formation of the intermediary product calcium bicarbonate which in turn is insoluble is a slow process.

Table 5. Changes in exchangeable Ca (cmol (+) kg soil^{-1}) under different liming materials over a period of 6 months

Liming materials	Mean exchangeable Ca	Ca after 6 months	Change in Ca over initial	Percentage Change in Ca over initial
Calcite	1.464	1.852	1.392	302.61
Gypsum	1.324	1.804	1.344	292.17
Dolomite	1.672	2.019	1.559	338.91
Calcium Silicate	1.078	1.340	0.88	191.30
Calcium oxalate	0.613	0.702	0.242	52.61
Absolute control	0.680	0.653	0.193	41.96
CD(0.05)	0.1969	0.540	-	-

Effect of depth of soil application

Though the application was done in surface and mixed well, the soil samples from four depths up to 60 cm were taken and analysed for pH and exchangeable Ca. The mean pH over the four different depths over the six months period with different quantities of the five different liming materials indicated significant difference among the four depths with 15-30 cm registering significantly the highest soil pH followed by 30-45 cm which was on par with 0-1 5cm. The pH recorded at a soil depth of 45-60 cm was significantly the lowest. As regards to the pH after six months, it is seen that, there was no significant difference among the four depths. However, the order of decrease in pH was 15-30, 0-15, 30-45 and 45-60 cm. Over the initial pH, 45-60 cm recorded the highest soil pH followed by 30-45, 0-15 and 15-30 cm (Table 6). Abure (2022) observed substantially high pH in subsurface soils of 20-40cm depth corroborating to our findings. However, the highest pH over initial seen in 45-60 cm soil depth can be justified based on the findings of Wakene (2001) under northeastern soils of Ethiopia.

Table 6. Changes in pH under different liming materials over a period of 6 months at different depths

Depth (cm)	Mean pH	pH after 6 months	Change in pH over initial	Percentage Change in pH over initial
0-15	5.50	5.995	0.745	14.19
15-30	5.729	6.046	0.706	13.22
30-45	5.535	5.942	0.902	17.90
45-60	5.242	5.844	1.224	26.49
CD(0.05)	0.1746	NS	-	-

The same trend as in the case of soil pH was seen with soil exchangeable Ca showing significantly the highest at 15-30 cm, which was on par with 0-15 cm followed by 30-45 cm. The pH at 45-60cm was significantly the lowest. After 6 months of application of treatments, the exchangeable Ca was highest at 45-60cm followed by 30-45cm, 15-30cm and 0-15cm. The increase over initial was maximum at 45-60cm and decreased in the order of increasing depth (Fig. 6). Abure (2022) also reported increase in exchangeable cations with increase in soil depth and the reason being the high clay content and accumulation of basic cations in the subsurface layer.

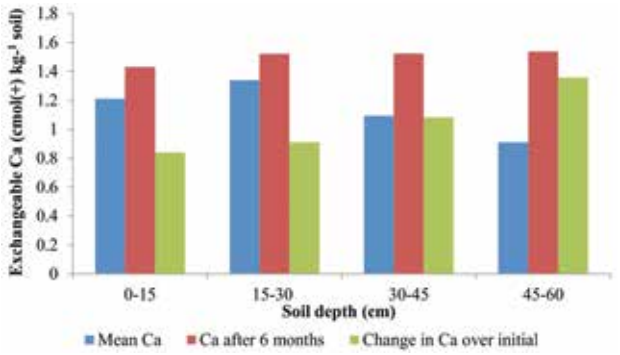

Fig. 6. Changes in exchangeable Ca under different liming materials over a period of 6 months at different depths

Our findings with different liming materials at various soil depths corroborates to the findings of Kisinyo et al., (2015) and Yorstand Ares, (2007) stating that, lime had no significant effect on soil pH, exchangeable Ca^{2+} and Al^{3+} in the subsoils at 20-40 and 40-60 cm depths and the reason being the slow solubility of lime and hence low mobility within the soil. The findings also conform to the report of Arya, (1990) that, very little changes in soil pH, exchangeable Ca^{2+} and Al^{3+} in the sub soil (12-85 cm depth) even after two and a half years after lime application at rates between 1.5-6.0 tons of lime per hectare.

Effect of quantity of application of liming materials

The mean pH over 6 months with different quantities of liming materials ranging from 0-1000g indicated significant effect on pH with significantly higher pH at

1000 g which was on par with 250,500 and 750 g of the liming materials. The significantly lower pH was noted with 50 g liming material, which was on par with 100,150, 200 g and control too. As regards to the pH after 6 months, it is seen that, 250 g resulted in significantly higher pH on par with 750 g followed by 500 g and 1000 g. There was no significant difference in pH among 50,100,150 and 200 g of the liming materials as they were on par in affecting the soil pH. Over the initial, 250 g caused the maximum increase followed by 750, 500 and 1000 g of the liming materials. Similarly, 250 g followed by 200 g and 100 g resulted increase in pH. The same trend was noted in the case of percentage increase in soil pH (Table 7).

Table 7. Changes in pH under various quantities of different liming materials over a period of 6 months at different depths

Quantity (g)	Mean pH	pH after 6 months	Change in pH over initial	Percentage change in pH over initial
0	4.955	5.345	0.285	5.63
250	5.913	6.624	1.564	30.91
500	5.811	6.176	1.116	22.06
750	5.844	6.593	1.533	30.30
1000	5.962	6.122	1.062	20.99
50	5.078	5.514	0.454	8.97
100	5.389	5.321	0.261	5.16
150	5.204	5.508	0.448	8.85
200	5.092	5.64	0.580	11.46
CD (0.05)	0.2315	0.4141	-	-

Though higher quantity resulted in an overall increase in pH, over the initial, the maximum increase was caused by the lowest quantity and this in turn conforms to the reports of Garbuio et al., (2011) in an Oxisol. Kisinyo et al., (2015) found that, lower lime rate (25% of the actual lime requirement) increased soil pH to highest peak in relatively shorter time and thereafter the pH began to decline compared to where half of the requirement was applied. This was likely because, Ca^{2+} ions in lower lime rate were depleted very fast, resulting in an increase in soil acidity. Similar results were reported by Sanchez (1976) who found that, the residual effect of lime depends on the amount of Ca^{2+} and/or Mg^{2+} ions still remaining in the liming material at any given time. They further indicated that, the exchangeable Ca^{2+} and Mg^{2+} increased with increasing lime rates while the exchangeable Al^{3+} decreased with increasing rates of lime.

As regards to exchangeable Ca, the mean over six months indicated significantly higher soil pH with 250 g of the liming materials on par with 500 g followed by 750 and 1000 g. Liming materials @ 50,100,150 and 200 g did not produce significant effect on soil exchangeable Ca. After 6 months of application, it was seen that, 250 g resulted in significantly higher exchangeable Ca on par with 750 g followed by 500 and 1000 g. The exchangeable Ca was significantly low with 50 g and was on par with 100 g, 150 g and 200 g. Over the initial 250 g caused the highest increase in exchangeable Ca, followed by 750, 500 and 1000 g. Similarly at the lower levels, 200 g followed by 250, 100 and 50 g caused increase in exchangeable Ca content of the soil and the same trend was followed in the percentage increase too (Fig. 7). The results indicated that, as the lowest rate of the liming material giving an exchangeable Ca content on par with the highest rates which may be attributed to the high available P content of the experimental soil (50-75 kg ha^{-1}) which in turn bound with phosphate making Ca unavailable as per the reports of Haynes and Ludecke (1981).

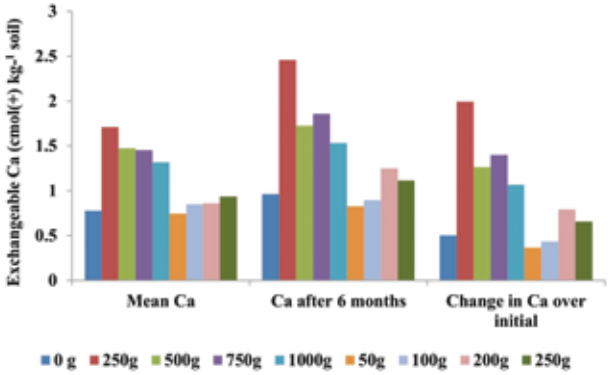

Fig. 7. Changes in exchangeable Ca under various quantities of different liming materials over a period of 6 months at different depths

Months after application of liming materials

The overall pH during the six intervals from initial indicated significant effect of months after application on soil pH with significantly higher at 6 MAA on par with 5 MAA followed by 4 MAA which was on par with 3 and 1 MAA and pH at 2 MAAwas on par with 1 MAA. As regards to the increase over initial as well as percentage

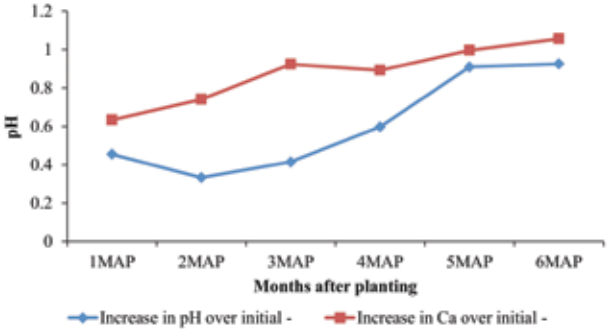

Fig. 8. pH and exchangeable Ca under two rates of different liming materials at different intervals at different depths

Table 8. Interaction effect of depth and quantity of liming materials on soil pH

Quantity (g) (Q)	Liming material	Depth (D) (cm)				Mean Q
		0-15	15-30	30-45	45-60	
0	Calcite, dolomite, gypsum	5.029	4.881	5.029	4.881	4.955
250		5.920	6.200	6.016	5.517	5.913
500		5.636	6.417	5.870	5.319	5.811
750		5.779	6.402	5.824	5.532	5.884
1000		5.823	6.335	6.125	5.565	5.962
50	Calcium silicate, Calcium oxalate	5.114	5.252	4.996	4.951	5.078
100		5.401	5.345	5.560	5.249	5.389
150		5.288	5.226	5.224	5.080	5.204
200		5.402	5.127	4.922	4.917	5.092
Mean (D)		5.567	5.850	5.616	5.289	
CD (0.05) D		0.1385				
CD(0.05) Q		0.2416				
CD(0.05) D × Q		0.3958				

increase over initial, the same trend was seen. As regards to the available Ca, there was significant difference among treatments and it is seen that, the exchangeable Ca at 6 MAA was on par with that at 5,4 and 3 MAA and the Ca at 1 MAA recorded significantly the lowest and the same trend was noted for both increase and percent increase over initial exchangeable Ca.

Interaction was noted for depth of sampling with quantity of application of liming materials for soil pH (Table 8). The pH observed was significantly higher for 500 g of the liming material at a depth of 15-30 cm which was on par with 250 g at 15-30 cm and 30-45 cm, 750 g at 15-30 cm and 1000 g at 15-30 cm and 30-45 cm. Absolute control at 15-30 and 45-60 cm recorded significantly lower soil pH which was on par with 50, 150 and 200 g of the liming materials at all depths and 100g at 45-60 cm depth. These results are in conformity with the findings of da Costa et al., (2016) that after 48 months, surface liming raised the pH of the soil in the surface layers to a depth of 0.20 m, with the same effect of spreading throughout the soil profile after 60 months of reapplying the lime.

Conclusion

One of the major consequences of soil acidity is the reduction in basic cations which are essential for plants resulting in phytotoxic concentrations of soluble aluminum affecting the growth and yield of crops especially in the case of crops like tannia which are very sensitive to subsoil acidity. To overcome this, liming of these types of soil is generally promoted as an effective management practice to increase soil pH, base cation concentrations, and ameliorate toxicity caused by aluminum and sometimes manganese. The present experiments were taken up both in controlled condition and field condition to see the best soil ameliorant for the acid laterite soils having subsoil acidity. Among the different soil amendments used in various quantities and based on the observations recorded on soil pH and exchangeable Ca over a period of 6-7 months from different soil depths ranging from 0-60 cm, it was seen that, there was drastic changes especially under field conditions at deeper soil layers for liming materials like calcite, dolomite, and gypsum. The study revealed more pH and Ca increase up to 30 cm soil depth with the minimum quantity of 250 g when compared to higher quantities as after saturation of the exchange sites with Ca, further substitution of Al with Ca was not occurring in the exchange sites. Hence, it is understood that the profitability of liming differed with liming rate, being more profitable at lower liming rates. However, under acidic soil conditions, specific to each crop and each soil type, more research is requiredfor an understanding of the appropriate lime rates to ensure overall profitability for producers and sustainable improvement of soil health.

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Study on compatibility of *Trichoderma asperellum* and fungicides for the development of environment friendly and cost-effective disease management strategies

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Abstract

Chemical fungicides are regularly being used to combat plant pathogens successfully. The escalating concern over human health and environmental safety often put pressure on farmers to lessen the use of chemicals. One of the approaches to minimize the use of fungicides is to integrate it with biological control agents (BCA). *Trichoderma* species are well known for their biological control activity against many plant pathogens. Application of *Trichoderma* is being endorsed for the management of diseases of tropical tuber crops viz., collar rot of elephant foot yam, tuber rot of cassava, stem and root rot of cassava, yam anthracnose and taro leaf blight. For an effective disease management, the activity of bio-control agent should not be stalled by the usage of fungicides. *Trichoderma asperellum* has been studied expansively as a BCA with results reliant on the specificity of the isolate. Present study was conducted at ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram to study the sensitivity of *T. asperellum* isolate tovarious fungicides. Knowledge onthe compatibility of possible bio agents with regularly used and newer agrochemicals is vital for refining/developingan efficient integrated disease management programme. Effect of twelve fungicideson mycelial growth of *Trichoderma* was tested by adopting poison food technique. Fungicides were tested at different concentrations ranging from 3.125 ppm to 3200 ppm. The fungicides, Copper oxychloride 50%, Cymoxanil 8%+ Mancozeb 64%, Mefenoxam 4%+ Mancozeb 64% and Cymoxanil 22.1% +Famoxadone 16.6% were compatible with *T. asperellum*at their recommended concentration by not inhibiting the mycelial growth. WhereasCarbendazim 50%, Carbendazim 25% + Mancozeb 50%, Hexaconazole 5%, Difenoconazole 25% and Metalaxyl–M 3.3%+ Chlorothalonil 33.1% inhibited the mycelial growth of bioagent indicating their incompatibility. A progressive increase in percent inhibition of radial growth in the fungus was observed as the concentrations of fungicides increased. The results obtained from the present study will help in revisiting integrated disease management strategies by combining bioagent, *Trichoderma* and fungicides for managing fungal diseases of tropical tuber crops.

Keywords: *Trichoderma asperellum,* Compatibility, Tuber crops, Fungicides

Introduction

In agriculture, fungicides play a pivotal role around the world and fungicide applications have been the key approach for plant disease management in many crops (Kongcharoen et al., 2020; Seni et al., 2018). However, disadvantages of fungicides have also been reported including residues on plant products and in the environment, deleterious effects on consumers and fungicide users and the environment, and the

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development of resistance to the fungicides (Steinberg and Gurr, 2020). Biocontrol agents (BCA) also take part in the management of plant diseases (Maheswary et al., 2020). Management of plant diseases by employing a potential BCA is always chosen over hazardous chemicals (Pandey et al., 2006). *Trichoderma*, a genus of diverse fungal species classified as anamorphic Hypocreales, have been studied extensively as BCA and have been developed into commercial products that are used worldwide (Kumar et al., 2014). *Trichoderma* has emerged as the most powerful biocontrol agent for management of soil-borne plant diseases (Kumar et al., 2013; Singh et al., 2017; Zaidi et al., 2018 and Kumar et al., 2020a). It demonstrates effective competition in challenging environments due to its natural resistance to antibiotics produced by competing microorganisms. Interaction between *Trichoderma* and plants not only enhances biomass and overall nutrition but also provides protection against various phytopathogens through direct mycoparasitic activity, competition for nutrients, or indirect induction of the plant defense system (Kumar et al., 2014). The use of suitable bio agents in conjunction with fungicides may improve disease control and management of plant diseases. The pathogens can be effectively managed if the fungicide used is compatible with the bioagents without causing any toxic effect (Papavizas and Lumsden, 1980). According to Maheswary et al. (2020), many chemicals inhibit the growth of *Trichoderma* spp. In integrated disease management (IDM), the activity of biological control agents should not be disadvantaged by the application of fungicides.

Key points in disease management are to adopt environment friendly approaches, hinder resistance development in pathogens against chemicals, promote antagonistic populations in the soil, and seek cost-effective disease management methods. Application of *Trichoderma* alone or in combination with chemicals is being practiced for the management of various fungal diseases of tropical tuber crops. *Trichoderma* not only suppress diseases but also offer growth promotion by improved seed germination, increased plant height and weight, root length, increased yield etc. Apart from the presently recommended fungicides, many other chemicals showed excellent activities against fungal/ oomycete pathogens causing diseases in tropical tuber crops. In this context, this study was aimed to assess the compatibility of *T. asperellum* with chemical fungicides for developing environment friendly and cost-effective disease management strategies against fungal diseases of tropical tuber crops.

Materials and Methods

Trichoderma culture

T. asperellum (ICAR- CTCRI T2) was isolated from the rhizosphere region of elephant foot yam plant, which was organically grown in the field of ICAR-CTCRI. The identity of the organism was confirmed as *T. asperellum* by ITS and TEF region amplification (gene bank accession number MN176380.1).

Fungicides

The fungicides recommended for managing the diseases of tropical tuber crops as well as few new fungicides, which inhibited pathogens of tuber crops *(in vitro*) were used for the study (Table 1).

Table1. Details of fungicides used for the study

Sl. No.	Brand Name	Technical Name/Active ingredient
1	Amistar	Azoxystrobin 23% (SC)
2	Folio Gold	Metalaxyl – M 3.3% + Chlorothalonil 33.1% (SC)
3	Equation Pro	Cymoxanil 22.1% + Famoxadone 16.6% (SC)
4	Score	Difenconazole 25% (EC)
5	Contaf	Hexaconazole 5% (SC)
6	Ridomil Gold R	Metalaxyl – M 4%+ 64% Mancozeb (WP)
7	Indofil M-45	Mancozeb 75% (WP)
8	Curzate	Cymoxanil 8% + Mancozeb 64% (WP)
9	Antracol	Propineb 70% (WP)
10	Tagstin	Carbendazim 50% (WP)
11	Blitox	Copper Oxychloride 50% (WP)
12	Sprint	Carbendazim 25% + Mancozeb 50 % (WS)

In vitro study on the effect of fungicides on mycelial growth

Compatibility was studied in terms of mycelial growth inhibition and the technique adopted was poisoned food technique (Zentmyer, 1955). Stock solutions of the fungicides were prepared in sterile distilled water. In case of combination fungicides, the content of the desired chemical in the fungicide was taken for calculating the quantity of the fungicide to be added. To begin the experiment, a concentration of 100 ppm was uniformly selected for all the fungicides. Required quantity of the stock solution was incorporated into sterile, molten, and cooled Potato Dextrose Agar medium (HIMEDIA) to get final concentration of 100 ppm. Fungicide amended medium was mixed gently and dispensed into Petri dishes.

T. asperellum was cultured on PDA for 48 h and mycelial discs (1cm diameter) were cut from the growing edges of the culture and placed in the centre of plates containing PDA amended with various chemicals at different concentrations. PDA plates inoculated with mycelial disc of *Trichoderma* without fungicide served as control.

Three plates were maintained for each concentration. The plates were incubated at 28±2°C. The growth of the colony was measured after 72 h. The radial growth of mycelium was measured at two points at right angle to each other from each of the three plates maintained for each concentration. The growth of the colony in control sets where no chemical was added was compared with that of various concentrations and the difference was converted into percent inhibition.

Based on the mycelial growth inhibition at 100 ppm, fungicides were grouped into two for further evaluation.The chemicals, which did not inhibit the growth of *Trichoderma* at 100 ppm were tested at higher concentrations, 200, 400, 800, 1600 and 3200 ppm. The chemicals, which inhibited growth at 100ppm were evaluated at lower concentrations of 50, 25, 12.5, 6.25 and 3.125 ppm. The percent inhibition of *Trichoderma* isolates was calculated based on the diameter of growth of the colony by using the formula of Vincent (1947).

$$I = \left(\frac{C-T}{C}\right) \times 100$$

Where, I is the per cent inhibition, C is the growth of *Trichoderma* isolates in control plates (without fungicide) and T is the growth of *Trichoderma* isolates in test plates (with fungicide).

Statistical analysis

The data on mycelial growth of *T. asperellum* at different concentrations of various fungicides was statistically analysed. Mean separation was determined according to Duncan's multiple range test ($p<0.05$)

Results and Discussion

The growth of *T. asperellum* was recorded at 24h interval until the mycelia growth in control plates completely covered the Petri plates (72 h). The results showed differential inhibitory action of the various fungicides towards mycelia growth of *Trichoderma*. At 100 ppm, seven out of twelve fungicides tested, did not inhibit the growthof *Trichoderma*. The seven fungicides, which did not affect the growth of *Trichoderma* were studied further at higher concentrations *viz*., 200, 400, 800, 1600 and 3200 ppm. At 200 ppm, maximum inhibition was in Azoxystrobin 23% (21.67%), whereas Propineb, Copper oxychloride, Cymoxanil + Mancozeb and Metalaxyl + Mancozebdid not inhibit the growth (Fig. 1a). Copper oxychloride was most compatible with *Trichoderma* and upto 400 ppm, it did not affect the mycelial growth. Other fungicides showed 15.56% to 30.11% inhibition (Fig. 1b). Even at 800ppm, copper oxychloride showed <5% inhibition only (Fig. 1c). However, at 1600 ppm, it showed >75% inhibition (Fig. 1d).

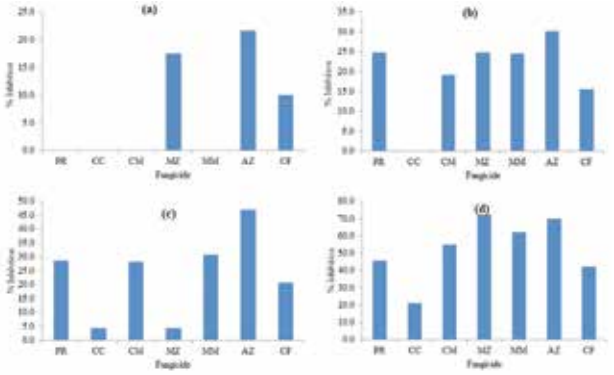

Fig. 1. Percent inhibition of mycelia growth at (a) 200, (b) 400, (c) 800 and (d) 1600 ppm of fungicides

*PR- Propineb 70% (WP); CC- Copper Oxychloride 50% (WP); CM- Cymoxanil 8% + Mancozeb 64% (WP); MZ- Mancozeb 75% (WP); MM- Metalaxyl – M 4%+ 64% Mancozeb (WP); AZ- Azoxystrobin 23% (SC); CF- Cymoxanil 22.1% + Famoxadone 16.6% (SC)

Table 2. Mycelial growth (cm) of *T. asperellum* on seven fungicides amended media (PDA)

Concentration (ppm)	Type of Medium						
	Propineb	Copper oxychloride	Cymoxanil 8%+ Mancozeb 64%	Mancozeb	Metalaxyl M 4%+ Mancozeb 64%	Azoxystrobin 23%	Cymoxanil 22.1% +Famoxadone 16.6%
100	9.00^{A}	9.00^{A}	9.00^{A}	9.00^{A}	9.00^{A}	9.00^{A}	9.00^{A}
200	9.00^{A}	9.00^{A}	9.00^{A}	7.43^{B}	9.00^{A}	7.05^{B}	8.10^{B}
400	6.77^{B}	9.00^{A}	7.27^{B}	6.77^{B}	6.80^{B}	6.29^{B}	7.60^{C}
800	6.43^{C}	8.60^{B}	6.47^{C}	5.53^{C}	6.23^{C}	4.77^{C}	7.13^{D}
1600	4.90^{C}	7.10^{C}	4.07^{C}	2.50^{D}	3.40^{D}	2.70^{D}	5.20^{E}
3200	4.57^{C}	0.10^{D}	0.10^{D}	2.00^{D}	2.97^{D}	2.23^{D}	2.67^{F}
Control	9.00^{A}	9.00^{A}	9.00^{A}	9.00^{A}	9.00^{A}	9.00^{A}	9.00^{A}
SE (d)	0.257	0.114	0.249	0.228	0.15	0.579	0.158
LSD at 1%	0.7846	0.3485	0.7619	0.6955	0.458	1.6393	0.4624

*Mean values with the same alphabet in the superscript did not differ significantly

The concentration of active ingredients in the combination fungicide, Cymoxanil 22.1% + Famoxadone 16.6% at recommended doseis 221 ppm (Cymoxanil) and 166 ppm (Famoxadone). The results of the study showed that the fungicide showed only 10% mycelial inhibition at 200 ppm. Hence, it is suitable for being used with the bioagent, *Trichoderma*. The fungicide Propineb, showed inhibition at 400 ppm (24.78%) and 28.56% inhibition was recorded at 800 ppm, which is above the recommended dose. However, even at the highest concentration tested (3200 ppm), Propineb did not completely arrest the mycelial growth. Propineb recorded least inhibition at 3200 ppm and inhibition was only around 50% (49.22%). This indicates its compatibility with *Trichoderma.*

At 100 ppm, *Trichoderma* showed sensitivity to 5 fungicides among the 12 studied. These five fungicides were separated and the sensitivity at lower concentrations was studied (Table 3). Carbendazim is most inhibitory to *Trichoderma* followed by combination fungicide, Carbendazim 25% + Mancozeb 50%. Even at the lowest concentration (3.125 ppm), these fungicides inhibited mycelial growth of *Trichoderma.* The recommended concentration is 500 ppm of the active ingredient. Hence, combination of these two fungicides with the biocontrol agent, *Trichoderma* is not recommended. Other three fungicides also inhibited the mycelial growth and none of them could support the mycelial growth at their recommended concentrations.

Integrated disease management (IDM) of soil-borne pathogens is the only way of reducing severe impact of chemical pesticides. Thus, studies on compatibility of *Trichoderma* to commonly available commercial pesticides matter very much in developing IDM modules (Kumar et al., 2020c). Based on the results of *in vitro* study, the 12 fungicides can be grouped into three based on their inhibitory effect on mycelial growth of *Trichoderma.* The compatible group involves the fungicides, Copper oxychloride 50%, Cymoxanil 8%+ Mancozeb 64%, Mefenoxam 4%+ Mancozeb 64% and Cymoxanil 22.1% + Famoxadone 16.6%.

The recommended dose of copper oxychloride is 1000 ppm (on active ingredient basis) and at the recommended dose, copper oxychloride and *Trichoderma* was compatible. Hence, copper oxychloride can be considered as a safe fungicide and recommended in IDM module for soil borne diseases in addition to biological control measures. Copper oxychloride (0.2%) was compatible and comparatively safer to *T. harzianum* and *T. viride* (Bagwan, 2010; Manandhar et al., 2020). According to Kumar et al. (2021), minimum inhibitory effect on growth and spore production was recorded with copper oxychloride, and hence can be used with *T. viride* in an integrated disease management practice for managing soil borne pathogens.

Similarly, at recommended dose, Cymoxanil 8% + Mancozeb 64% (WP) and 4% Metalaxyl – M 64% Mancozeb (WP), the contents of Cymoxanil and Metalaxyl were 80 ppm and 40 ppm, respectively. These chemicals at 200 ppm did not inhibit the growth of *Trichoderma* and are also compatible with *Trichoderma* at their recommended concentrations. Least inhibitory effect of Metalaxyl M-4%+ Mancozeb 64% has been reported by many workers (Desai and Kulkarni, 2004; Thoudam and Dutta, 2014; Theerthaet al., 2017; Manjunath et al., 2018 and Manandhar et al., 2020). Compatibility of Mancozeb and *Trichoderma* (Bagwan, 2010; Manjunath et al., 2018; Manandhar et al., 2020) and Cymoxanil 8% + Mancozeb 64% and *Trichoderma* had been reported (Manjunath et al., 2018; Manandhar et al., 2020).

The moderately compatible group includes, Mancozeb, Propineb and Azoxystrobin 23%. Azoxystrobin was highly compatible with *T.harzianum* and *T. viride* with no inhibition (Shashikumar et al., 2019; Manjunath et al., 2018), However, Maheshwary et al., (2020) reported 38.14% inhibition with 100 ppm Azoxystrobin. Propineb was fully compatible with *T. viride* (Madhavi et al., 2011). Theertha et al., (2017) found that Mancozeb at 100 ppm showed 23.0% inhibition and the inhibition rate is positively correlated with the strength of the fungicide.

The incompatible fungicides were Carbendazim 50%, Carbendazim 25% + Mancozeb 50%, Hexaconazole 5%, Difenoconazole 25% and Metalaxyl–M 3.3%+ Chlorothalonil 33.1%. Carbendazim either alone or in mixture proved to be highly toxic to *T. viride*, or should

Table 3. Mycelial growth (cm) of *T. asperellum* on the five fungicides amended media (PDA)

Concentration (ppm)	Metalaxyl-M 3.3%+ Chlorothalonil 33.1% (SC)	Difenconazole 25% (EC)	Hexaconazole 5% (SC)	Carbendazim 50% (WP)	Carbendazim 25% + Mancozeb 50 % (WS)
100	2.30	2.00	0.00	0.00	0.00
50	3.40	2.96	0.00	0.00	0.00
25	5.80	4.50	0.00	0.00	0.00
12.5	7.90	6,70	4.25	0.00	0.00
6.25	9.00	9.00	7.30	0.00	0.00
3.125	9.00	9.00	9.00	0.00	1.07
Control	9.00	9.00	9.00	9.00	9.00

not be combined for seed or soil application along with bio-control agents (Theertha et al., 2017; Dethoup et al., 2022). Growth and sporulation of *T. viride* was totally inhibited by Carbendazim, Hexoconazole, Carbendazim + Mancozeb at all the concentrations (Kumar et al., 2021). Non compatibility of *Trichoderma* spp. with Carbendazim fungicides had been reported by many workers (Sonavane and Venkataravanappa, 2017; Kumar et al., 2018). The high inhibition of benzimidazole compounds like Carbendazim is due to its binding with ß-tubulin of fungal pathogen causing inhibition of microtubule assembly which ultimately hinders cell division and may lead to cell death (Zhou et al., 2016).

Hexaconazole's high inhibition capability is due to the presence of systematic demethylation inhibitors. These inhibitors primarily target the vegetative stage of fungi, disrupting the development of mycelium both internally and externally within the host plant (Khalfallah et al., 1998).

The percent of compatibility decreased with an increase in the concentration of fungicide. Reduced amount of fungicide can weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonist. Therefore, rather than applying these chemicals alone, it is imperative to use *Trichoderma* in combination with fungicides at the lower concentration for effective management of fungal pathogens since they do not have a side effect on the environments. Like any other crops, IDM is practiced in tuber crops also for the management of various diseases. In case of taro, cormel treatment with *Trichoderma* and spraying with Metalaxyl+ Mancozeb or Mancozeb are being recommended to combat taro leaf blight caused by *Phytophthora colocasiae*. Result of the study indicated their compatibility and endorses the integration of chemical and biocontrol for the effective management. However, for the management of diseases like collar rot in elephant foot yam and anthracnose in greater yam, corm treatment with *Trichoderma* and Carbendazim or Carbendazim + Mancozeb forms part of IDM. The present study clearly establishes the non-compatibility of *Trichoderma* with the chemicals.

Conclusion

In sustainable agriculture, improved crop production technologies coupled with plant protection strategies play a vital role in plant disease management thereby enhancing the production and productivity of the crops. Considering the sustainability of the ecosystem and the ever-increasing concern over the presence of toxic chemicals in the food chain, lessening the use of chemicals has become an essential step. The combination of chemicals and bio-agents can take care of these concerns. The compatibility of these strategies needs to be ascertained before making recommendations to the farming community. The present study confirms the compatibility of *T. asperellum* with various agrochemicals is matching with compatibility profile of other species of *Trichoderma* viz., *T. viride* and *T. harzianum*. The class of fungicides, which were classified as compatible or moderately compatible can be recommended or continue to recommend in IDM practices. The present recommendations involving Carbendazimand its combinationshave to be revisited. Being a fungus, *Trichoderma* is affected by many of the fungicides. Hence utmost care may be taken while applying incompatible combination of fungicides and bio-agents or a safe interval must be provided. To draw final conclusions, the effects of agrochemicals on *T. asperellum* must be investigated under field conditions.

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Insecticide residues in tuber crops and its effect on soil microbes

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Abstract

Sucking pests are one of the most important problems in tuber crop cultivation. For the management of sucking pests' whitefly, mealybug, scale *etc*., systemic insecticides like imidacloprid and dimethoate are recommended for drenching and spraying. In the present study, these insecticides were tested for their impact on soil micro-organisms and for their presence in cassava.Beneficial soil microbes *Bacillus cereus*, *Beauveria bassiana* and *Trichoderma* spp. were isolated from rhizosphere soil of tuber crops and their growth in insecticide applied media were compared. Residue analysis of the plant samples (cassava leaves and tubers) was conducted after the application of insecticides at recommended doses to the plants. Observations were taken after 1, 7, 14 and 30 days of application. From the LC-MS and GC-MS study, it was found that tuber (edible part) is safe from imidacloprid residue even 24 h after the application. But, 0.051 ppm dimethoate residue was noticed in cassava tubers after 30 days of drenching with the insecticide.

Keywords: Insecticide residue, Tuber crops, Soil microbes, Dimethoate, Imidacloprid

Introduction

The term pesticide encompasses a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others. Among them are organochlorine insecticides (OCs), which have been used successfully to control a number of diseases such as malaria and typhoid and were banned or restricted in most technologically advanced countries after the 1960s (Pathak et al., 2022). The introduction of other synthetic insecticides, organophosphate insecticides (OP) in the 1960s, carbamates in the 1970s and pyrethroids in the 1980s, contributed significantly to pest control and agricultural production. Ideally, a pesticide must be lethal to the target pests but not to non-target species, including humans. Unfortunately, this is not the case, which is why the controversy over the use and misuse of pesticides has come to the surface. The widespread use of these chemicals, true to the saying, 'if little is good, a lot more will be better' is having devastating effects on humans and other life forms (Aktar et al., 2009).

Soil microorganisms are the most abundant biota in soil and are responsible for nutrient and organic matter cycling, soil fertility, soil remediation, plant health and primary production of the ecosystem. Beneficial microorganisms include those that form symbiotic associations with plant roots (rhizobia, mycorrhizal fungi, actinomycetes, diazotrophic bacteria), promote nutrient mineralization and availability, produce plant growth hormones, and are antagonists of plant pests, parasites, or diseases (biocontrol agents). Many of these organisms already occur naturally in the soil, although in some situations it may be beneficial to increase their populations either through inoculation or through

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the use of various agricultural management techniques that increase their abundance and activity. Although microbes are ubiquitous on Earth, their distribution across different habitats is not uniform. Microbial populations are usually found in nutrient-rich niches with a constant supply of easily usable nutrients, such as the rhizosphere. The rhizosphere has an enormous pool of soil microorganisms and is considered a hotspot for microbial colonization and activity. It is the largest ecosystem on earth with enormous energy flow (Barriuso et al., 2008).

Bacteria are the most abundant microbes in the rhizosphere and have a significant impact on the plants growing there. Up to 15% of the total root surface can be covered by different bacterial strains (Van Loon, 2007). The most common bacterial genera reported in the rhizosphere are *Bacillus* and *Pseudomonas*. Different *Bacillus* strains represent the most important Gram-positive inhabitants of the rhizosphere (up to 95% of all Gram-positive soil bacteria) (Barriuso et al., 2008). *Bacillus* can form endospores and produce antimicrobial substances that inhibit other competitors. Representatives of the genus *Bacillus* are increasingly used in agriculture to promote plant growth and to protect against plant pathogens (Qiaoet al., 2017). Antifungal agents are widely used for biological control of both plant fungal diseases and insect pests. Various non-pathogenic (saprophytic) strains of *Trichoderma* spp. have been used to reduce damage (root rot, wilt, desiccation, and bald patches) caused by other pathogenic fungi (e.g., *Pythium, Sclerotium, Verticillium*) (Cook, 1994). According to Mascarin et al. (2019), fungal entomopathogens like *Beauveria* are very good options against (biocontrol agents) arthropod pests. Also, many researchers (Barreto et al., 2004; Imoulan and Elmeziane, 2014; Amnuaykanjanasin et al., 2013) underlined the role of *B. bassiana* as potent entomopathogens.

Over the last 50 years, there has been an increasing use of pesticides in the environment. The ideal pesticide should be toxic only to the target organisms, be biodegradable, and not leach into groundwater. In the European Union, approval systems for new pesticides are governed by common guidelines (Lynch, 1995), which state that effects on microbial processes should be measured by testing a sensitive soil that represents a worst-case scenario. Such effects of pesticides in the environment have classically been studied using functional parameters such as microbial activities in the soil (Greaves, 1982), which are important to hold onto soil nutrients also (Savonen, 1997). Insecticide residues in agricultural products are of great concern due to their potential impact on human health, the environment and food safety. Several types of insecticides are used in agriculture, including organophosphates, pyrethroids, neonicotinoids, and more. Each has different chemical properties and modes of action, which can influence their persistence in the environment and the likelihood of residues on crops. Many countries have established regulations and MRLs for insecticide residues in food. These limits ensure that the level of residues on agricultural products is within safe and acceptable levels for human consumption. Violation of these limits may result in product recalls or restrictions on the sale of products. Research has led to the development and improvement of analytical methods for the detection and quantification of insecticide residues in foods. High-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) are commonly used techniques.

The management of insecticide residues for soil health and safe agricultural produces is a multifaceted issue involving public health, environmental protection, and sustainable agriculture. Ongoing research and the adoption of best practices are essential to ensure food safety, protect the environment, and safeguard public health.

Materials and Methods

Materials

Rhizosphere soil was collected from tuber crop fieldsof Thrissur, Idukki and Thiruvananthapuram districts of Kerala, to know the impact of insecticides on important beneficial soil microbes. For culturing bacteria, nutrient agar and for fungi, Rose Bengal Agar (initial screening)/ *Potato Dextrose Agar* (sub-culturing) media were used. Insecticides imidacloprid 17.8% SL and dimethoate 30% EC are used in the study, as these are the most common ones used against sucking pests of tuber crops. For residue analysis LC-MS and GC-MS were employed.

Methods

Soil collected from rhizosphere of tuber crops and uncultivated soil were taken in earthen pots with 30 cm × 30 cm size. Rhizosphere soils were treated with different concentrations of imidacloprid (0.3, 0.5 and 1.0 ml l^{-1}) and made three replications each. Similarly, for dimethoate also three concentrations (1.5, 2.0 and 2.5 ml l^{-1}) were used. The soils were sieved to get rid of pebbles and other larger particles. One grams of the fine soils were weighed using weighing balance. Serial dilution was done with 1gm of above samples mixed with 9ml of distilled water (10^{-1}). Mixed well and made serial dilutions up to 10^{-6}.

i. Enumeration of microbial population

Bacteria: One ml of samples from 10^{-6} dilutions were poured into sterile Petri plates using pipette. Nearly 15 ml of molten nutrient agar was poured over the sample and mixed gently. After the media were solidified, the plates were incubated for 24-48 h. Three replicas were maintained.

Fungi: One ml of samples from 10^{-4} dilutions were poured into sterile Petri plates using pipette. Nearly 15 ml of molten Rose Bengal Agar was poured over the sample and mixed gently. After the media were solidified, the plates were incubated for 48-72 h. Three replicas were maintained.

Subculture of promising microorganisms: After the incubation, the growth of the colonies of microorganisms were observed. The colonies were differentiated based on their morphology. The same procedures were repeated for bacteria and fungi. The dissimilar colonies were then inoculated to different test tubes for the purpose of sub culturing (nutrient agar medium for bacteria and potato dextrose agar medium for fungi).

To study the effect of insecticides on bacteria, 60, 100 and 200 μl imidacloprid (@ 0.3, 0.5, 1.0 mlL^{-1}) was added to 200 ml of nutrient agar taken in 500 ml conical flasks. Also, 300, 400 and 500 μl dimethoate (@ 1.5, 2.0 and 2.5 mlL^{-1}) was added to 200 ml of nutrient agar. Agar plates without adding insecticides were taken as control. Streaking was done with selected bacteria having promising growth. Growth was observed after 24 h. Bacterial identification was by using NCBI blast, after DNA isolation, PCR and Sanger sequencing. To study the effect of insecticides on fungi, 60, 100 and 200 μl imidacloprid (@ 0.3, 0.5, and 1.0 ml l^{-1}) was added to 200 ml of PDA taken in 500 ml conical flasks. Again, 300, 400 and 500 μl dimethoate (@ 1.5, 2.0, and 2.5 ml l^{-1}) was added to 200 ml of PDA taken in 500 ml conical flasks. PDA plate without adding insecticides were taken as control. Plating was done with selected promising fungi and observed the growth after 48 h. Fungi were using NCBI BLAST after Sanger sequencing.

ii. Residue analysis in cassava plant parts

Insecticides, imidacloprid 17.8% SL and dimethoate 30% EC were taken at different doses. Imidacloprid was taken at doses 0.3 ml l^{-1}, 0.5 ml l^{-1}, 1.0 ml l^{-1}, whereas dimethoate was used at 1.5 ml l^{-1}, 2 ml l^{-1}, 2.5 ml l^{-1}. These insecticides were treated by both spraying and drenching in 5-month-old cassava plants. The plant samples (cassava leaves and tubers) were collected after 1, 7, 14 and 30 days. Residues in the plant parts were detected using LC-MS and GC-MS.

Results and Discussion

Effect of insecticides on microbial growth

After adding insecticides imidacloprid and dimethoate at recommended doses in nutrient agar medium, growth of *Bacillus cereus* was compared. The same procedure was followed for the growth of *Trichoderma* sp. and *Beauveria bassiana* in PDA plates. After inoculation and incubation of the bacteria into Petri plates containing insecticides, it was observed that *Bacillus* growth was comparable in control and imidacloprid added plate, whereas very poor growth/ no apparent growth was observed in dimethoate added one (Fig. 1). Similarly, from the PDA plates it was observed that *Trichoderma* growth was not found in plates containing dimethoate at different concentrations. But growth was detected in plates containing imidacloprid, even though it was lesser compared to control (60 per cent of control's growth after 48 h)(Fig. 2). The trend was similar in case of *Beauveria* also.

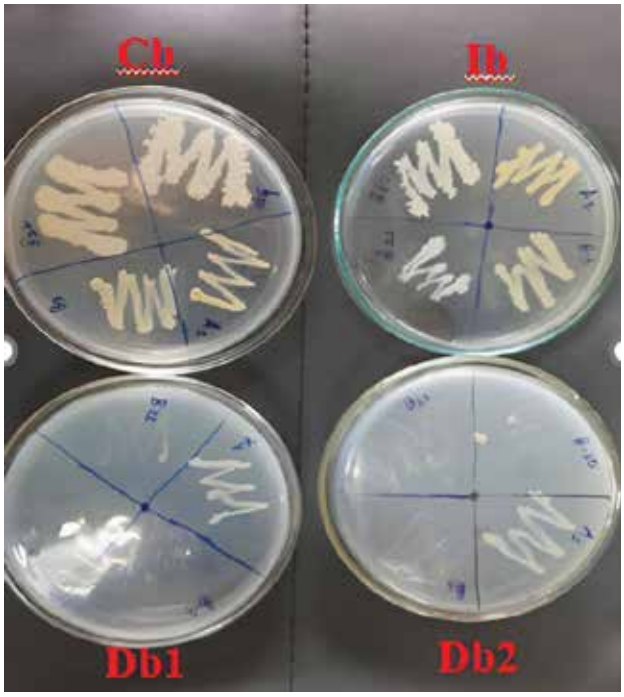

Fig. 1. *Bacillus* growth in nutrient agar plate (Ib-imidacloprid added medium,Cb-Control, Db 1 & Db 2- dimethoate added medium)

Heavy treatment of soil with pesticides can cause populations of beneficial soil microorganisms to decline. According to soil scientist Dr. Elaine Ingham 'Soil deteriorates when we lose both bacteria and fungi'. The excessive use of chemical pesticides has similar effects on soil organisms as the excessive use of antibiotics in humans. Indiscriminate use of chemicals may work for a few years, but after a while there are no longer enough beneficial soil organisms to hold onto the nutrients (Savonen, 1997).

Bacillus are ubiquitous in nature (Parker and Duerden, 1990) and a number of members of the genus *Bacillus* are natural agents for biological control of invertebrate pests and are the bases of many biological commercial insecticides (Molina et al., 2010). Studies by Emmert and Handelsman (1999), states that interaction of *B. cereus* with the host plant revealed some promising avenues for improving biocontrol. *Trichoderma* can not only prevent diseases, but also promote plant growth, improve nutrient utilization efficiency, increase plant resistance, and improve environmental pollution caused

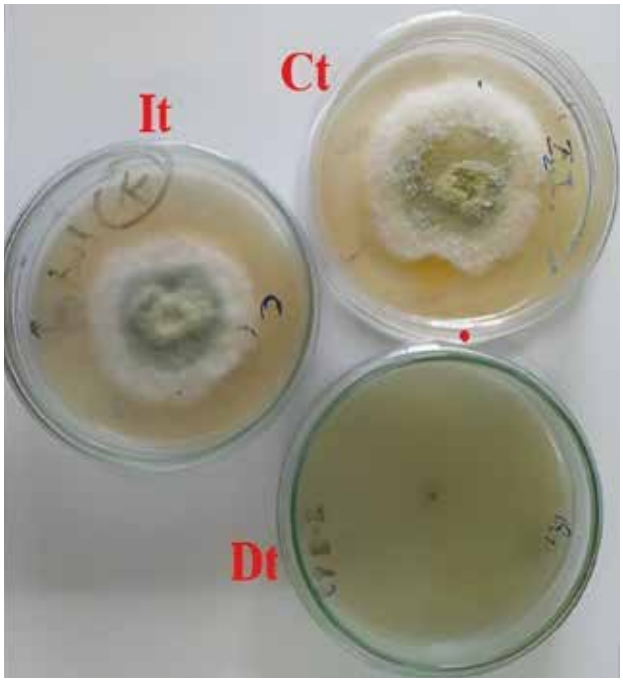

Fig. 2. *Trichoderma* growth in PDA plate (It- imidacloprid added medium, Ct- Control, Dt- dimethoate added medium)

by agrochemicals. Barreto et al. (2004), Imoulan and Elmeziane (2014) and Amnuaykanjanasin et al., (2013) emphasized the role of *B. bassiana* as potent entomopathogens. Based on the study conducted by Dara (2017), *B. bassiana* is compatible with many chemical fungicides. For the control of nymphs and adults of whitefly (*B. tabaci*), among entomopathogenic fungi, *B. bassiana* found to be one of the best options (Harish et al., 2019). Based on the literature available about the potential of soil microbes, *B. cereus, Trichoderma* and *B. bassiana* were used for the study of effect of insecticides on them.

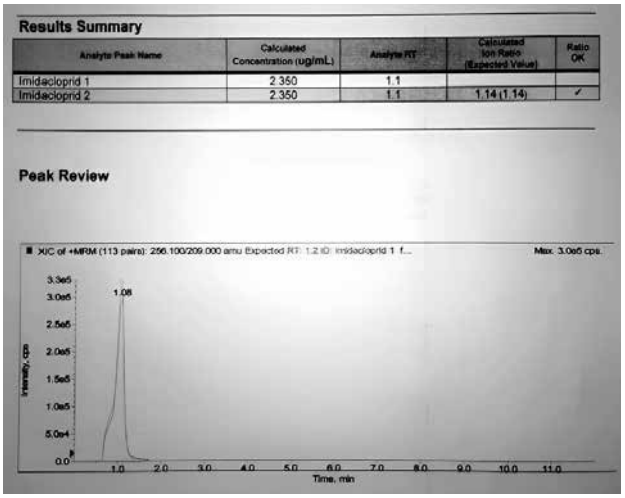

Fig. 3. LC-MS study result of imidacloprid residue in cassava plant 24 h after treatment

Many studies were conducted to realize the persistence of imidacloprid. The works of Juraske et al., (2009), Donnarumma et al., (2011) and Mohapatra et al., (2012) revealed not much of residual toxicity above MRL both in plants and soil for the insecticide. Butin case of dimethoate, like in the present study, found to cause many adverse effects in non-target organisms (Van Scoy et al., 2016). The study conducted by Getenga et al., (2000) shows that dimethoate is highly persistent in soil.

Residue of insecticides in cassava

Imidacloprid residue was detected only in the case of spraying, not in drenching and that too in leaves only. The quantity observed was 2.35 ppm after 24 hours of treatment. Residue was not detected in LC-MS study, for IT1D, IT1S and IL1D (Table 1 & Fig. 3). GC-MS study for dimethoate showed, at a spraying dose of 2.5 mlL^{-1}, 4.63 ppm of residue was present in leaves after 24 h of treatment. In the same dose, 0.051 ppm of dimethoate residue was noticed in cassava tubers 30 days after drenching (Table 2 & Fig. 4).

Table 1. Imidacloprid residue at various parts of cassava plants one day after treatment (LC-MS)

Sl. No.	Sample code	Identification code*	Pesticide detected	Residue (ppm)
1	RF/0231/03/19	IT1D	Nil	Nil
2	RF/0232/03/19	IT1S	Nil	Nil
3	RF/0233/03/19	IL1D	Nil	Nil
4	RF/0234/03/19	IL1S	Imidaclo-prid	2.35

*(I-imidacloprid, T-tuber, L-leaf, D-drenching, S-spraying)

According to the study, dimethoate is highly persistent in cassava tubers (@ 2.5 mlL^{-1}, even after 30 days of drenching. In the present study 0.051 ppm dimethoate could be detected using GC-MS study in tubers (Table 2 & Fig. 4). According to European Commission standards (ECS), 0.01 ppm is the safe limit for dimethoate in food crops (European Commission Standards on food crops,

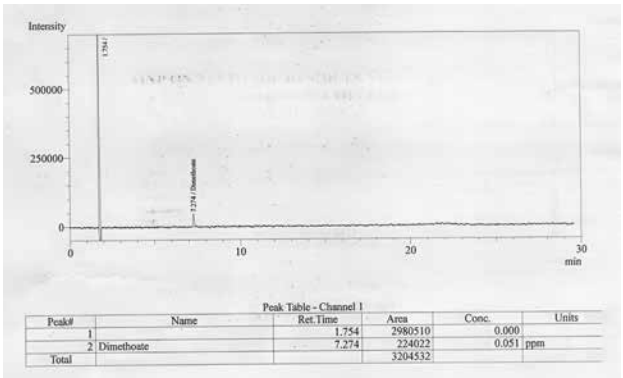

Fig. 4. GC-MS study result of dimethoate residue in cassava tuber 30 days after treatment

Table 2. Dimethoate residue at various parts of cassava plants until 30 days after treatment (GC-MS)

Sl.No.	Sample code	Identification code	Pesticide detected	Residue (ppm)
1	RF/0235/03/19	DT1D	Nil	<LOQ*
2	RF/0236/03/19	DT1S	Nil	<LOQ
3	RF/0237/03/19	DL1D	Nil	<LOQ
4	RF/0238/03/19	DL1S	Dimethoate	4.63
5	RF/0255/03/19	DT14D	Nil	<LOQ
6	RF/0256/03/19	DT14S	Nil	<LOQ
7	RF/0257/03/19	DL14D	Nil	<LOQ
8	RF/0258/03/19	DL14S	Nil	<LOQ
9	RF/0259/03/19	Control (Tuber)	Nil	<LOQ
10	RF/0260/03/19	Control (Leaf)	Nil	<LOQ
11	RF/0274/03/19	DT30D	Dimethoate	0.051
12	RF/0275/03/19	DT30S	Nil	<LOQ
13	RF/0276/03/19	DL30D	Nil	<LOQ
14	RF/0277/03/19	DL30S	Nil	<LOQ

*LOQ (Limit of Quantification) = 0.05 ppm
(D- dimethoate, T-tuber, L-leaf, D-drenching, S-spraying)

2022). In case of Imidacloprid, residue above MRL could not be detected after one week both in leaves and tubers but detected after 24 h (Table 1 & Fig. 3). The residual problem of dimethoate was observed in drenching not in spraying. So, we can say even if someone use the pesticide, try not to go for drenching and can opt for spraying in shoots.

Likewise, dimethoate toxicity and safety of imidacloprid was explained by Rehberg et al., (2022) in his study. According to El-Sheikh et al., (2022), a study conducted in Egypt shows that dimethoate is above the MRL in many fruits and vegetables collected from farmers' markets and may cause many potential health risks to humans.

Conclusion

Imidacloprid is found to be safe against beneficial soil microbes compared to organophosphorus insecticide, dimethoate. Also, imidacloprid is comparatively innocuous to use in cassava at recommended dose, whereas, dimethoate may only be used in spraying, but not for drenching in cassava and other tuber crops.

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Change in climate and climate suitability of major taro [*Colocasia esculenta* (L.) schott] growing regions of India

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Abstract

Root and tuber crops such as taro [*Colocasia esculenta* (L.) schott] play a vital role in food security and livelihoods and yet neglected in climate change impact studies and large-scale crop improvement programs. This study attempts ensembled multi-model prediction of change in climate and climate suitability of taro in major taro growing regions of India by 2030 and 2050 under 4.5 and 8.5 representative concentration pathways (RCP). Climate, suitability (EcoCrop model) and suitability changes were analysed using Arc GIS 10.1 and Diva GIS 7.5. According to the study, under RCPs 4.5 and 8.5, the major taro growing regions will experience warming of the climate by 2030 and 2050. The mean temperature of major taro growing regions in 2030 will increase by 1.15- 1.49°C and 1.58 – 2.09°C for RCPs 4.5 and 8.5; and 1.35 - 1.70°C and 2.02 - 2.68°C for RCPs 4.5 and 8.5 in 2050. The precipitation in 2030 will increase by -2.01 – 82.07 mm and 2.84 - 128.02 mm for RCPs 4.5 and 8.5; and in 2050 it will change by 13.48 to 16.98 mm and 1.09 to 108.54 mm for RCPs 4.5 and 8.5. The climate suitability will change by -12.31 to 5.17% and -14.29 to 7.63% in 2030 for RCPs 4.5 and 8.5; and -18.26 to 6.57% and -24.1 to 9.39% for RCPs 4.5 and 8.5 in 2050.

Keywords: Taro, Climate change, Climate suitability, Representative concentration pathways, EcoCrop model

Introduction

Feeding a growing global population in a changing climate presents a significant challenge to society (Ericksen et al., 2009). Numerous issues, including rising demand, greater input costs, soil degradation, need to reduce greenhouse gas emissions, and increasing competition for land and water from non-food uses have an impact on food security (Hertel, 2011). Additionally, it is anticipated that yields will be impacted by climate change greatly (Tubiello et al., 2007). Root and tubers are the second most important group of food crops in the developing world after cereals, contributing to diets of over 2 billion people across the tropics and subtropics with annual production of about 800 million tons. They are crucial for the rural people's supply of calories and nutrients and help to lower food insecurity and malnutrition (Tadele, 2019), and it includes cassava, sweet potato, cocoyam and yams. These roots and tubers, however, are underexploited as there is a lack of genetic variety, biotechnology applications, and scientifically valid assessments of their adaptability (Mabhaudhi et al., 2019). Due to this, many root and tuber crops, including cocoyam, are categorized as neglected and under-utilized crops (Tumuhimbise, 2015).

Taro [*Colocasia esculenta* (L.) Schott], is an annual herbaceous plant belonging to the family Araceae (Prajapati et al., 2011) and is cultivated in 50 countries with 88.69% production concentrated in Africa.

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Globally, taro (cocoyam) is cultivated in an area of 1.79 m ha with a total production of 12.37 m tons and the average productivity is 6.91 t ha^{-1} (www.fao.org/faostat). According to Gananca et al. (2015), taro has a high moisture demand and thrives on the edges of wet fields and next to streams. It prefers regions with significant annual rainfall (>1,500 mm) and even distribution (Mwenye 2009). Lim (2015) claims that low rainfall inhibits corm growth because occasional moisture stress results in low yields, corms with a dumbbell form, and corms of poor quality. Addressing temperature, it can withstand maximum temperatures of at least 21°C and minimum temperatures of at least 10°C. As a result, it is colder tolerant than other tuber crops because it can withstand temperatures as low as 10°C. However, freezing temperatures harm the leaves and reduce yield (Lim 2015; Raemaekers, 2001). According to the FAO (1994), the crop matures over a long period of time between 180 and 300 days.

The most widely used tools to study the climate suitability changes of crops is EcoCrop model (Hijmans et al., 2001; Beebe et al., 2011; Jarvis et al., 2012; Vermeulen et al., 2013; Villegas et al., 2013; Sabitha et al., 2016; Piikki et al., 2017, Remya et al., 2018, Shiny et al., 2019). The basic mechanistic model (EcoCrop) implemented uses environmental ranges as inputs to determine the main niche of a crop and then produces a suitability index as output. The model was originally developed by Hijmans et al., (2001) and named EcoCrop since it was based on the FAO-EcoCrop database. The impact of future climate on suitability of taro in India is not yet studied by EcoCrop model. Hence, the present study was aimed at understanding whether taro really is a crop of merit for adaptation to climate change. Hence, this study used ten different coupled global climate models (GCMs) of the coupled model intercomparison project (CMIP5) to predict changes in the future climate and the climate suitability of taro in the major growing environments of India at two representative concentration pathways (RCP) of 4.5 and 8.5 by 2030 and 2050.

Materials and Methods

Study area

The study area included current taro growing regions of India. The presence point map of taro in India was developed according to expert knowledge of scientists working in ICAR-CTCRI and AICRP-TC (All India Coordinated Research Project on Tuber Crops) centers and based on available literature, identified principal regions where taro is cultivated currently. The geographic coordinates of these regions at 30 seconds spatial resolution using the district boundary shape file of each growing area (Fig. 1) were extracted and a total of 64,393 coordinates as taro presence points were obtained covering 10 states (157 districts) in India, *viz.,* Andhra Pradesh, Assam, Bihar, Kerala, Meghalaya, Rajasthan, Tamil Nadu, Telangana, Uttar Pradesh and West Bengal.

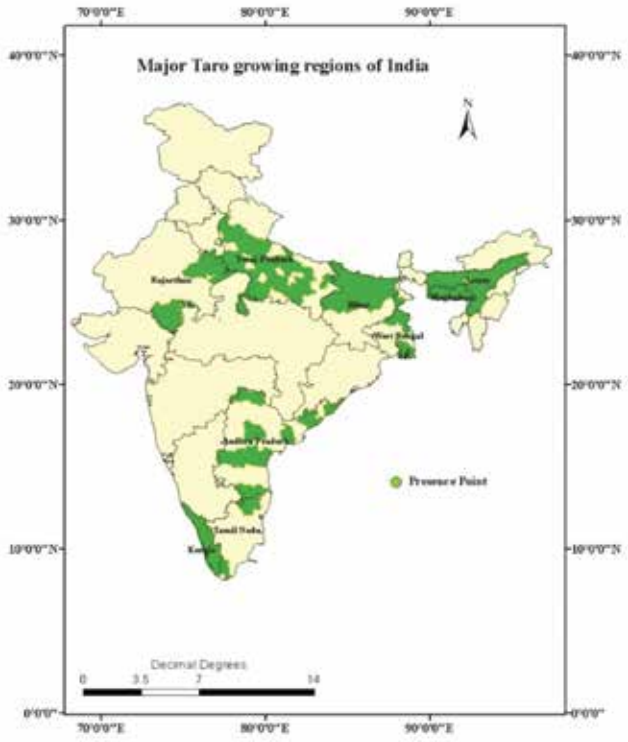

Fig. 1. Presence points map of major taro growing regions in India

Current climate data

The spatial data of current climate were obtained from the WorldClim dataset (Hijmans et al., 2005; http://www.worldclim.org) at the spatial resolution of 30 arc-second equivalent to about 0.86 km^2 at the equator, commonly referred to as '1- km' resolution (Hutchinson, 1995) to depict current climatic conditions. The data include monthly time series of minimum, maximum and mean temperature and precipitation. The WorldClim spatial dataset was developed using data from ~ 47,000, 23,000 and 13,000 weather stations (globally) for monthly information on precipitation, mean temperature and diurnal temperature range data respectively. The data was processed using a quality checking algorithm and then developed a continuous climate surface using thin plate spline algorithm (Hutchinson and Hoog, 1985; Hijmans, 2003), with elevation, latitude and longitude as independent variables. (http://www.ccafs-climate.org/data). The database includes precipitation, mean temperature, minimum and maximum temperature from 47554, 24542 and 14835 locations respectively (Challinor et al., 2010).

Future climate data

Representative Concentration Pathways (RCPs) are essential for assessing potential future climate changes and their impacts. It is used in climate modeling to project

future greenhouse gas concentrations and associated radiative forcing (Vuuren et al., 2011). The downloaded future climate data included monthly time series data of maximum, minimum and mean temperature and total monthly precipitation for 4.5 and 8.5 Representative Concentration Pathways (RCP) for 2030 and 2050 from 10 different coupled global climate models (GCMs) of coupled model inter-comparison project 5 (CMIP5) (Table 1) used in the IPCC Fifth Assessment Report (AR5) (Pravat et al., 2015). The model selection was based on the availability of data for both RCPs 4.5 and 8.5 for 2030 and 2050 and also based on optimum model ranking for climate change projections for Indian monsoon precipitation (IPCC, 2014) considering that the models will be more precise to predict future suitability and suitability change of taro in India.

The emission scenarios were based on total anthropogenic radiative forcing at the end of the 21st century. Different paths were taken by the economic models to reach four different radiative forcing that were correspondent to different concentration paths of the green house gases, the so-called RCPs. Considering the possibility that green house gas emission will be at an intermediate level in future due to various strategies and considering the future hidden threat from uncontrolled high green house gas emissions, the two green house gas emission scenarios of RCPs 4.5 and 8.5 were considered. RCP 4.5 corresponds approximately to B1 scenario in fourth assessment report of IPCC (AR4); the radiative forcing grows almost linearly up to about the year 2060 and then slows down the growth rate until the end of the century where it stabilizes. The radiative forcing in RCP 8.5 corresponds approximately to A2 scenario in AR4, it grows almost linearly during the 21st century, but with higher radiative forcing values (Sin et al., 2014). The RCP 4.5 corresponds to the radiative forcing of 4.5 Wm^{-2} and RCP 8.5 to 8.5 $Wm^{-2.}$ This study did not account for the carbon dioxide fertilization effects in the simulations. The spatially downscaled (delta method) GCM predicted future climate data were downloaded from http://www.ccafs-climate.org/data. The model resolution was 30 arc seconds.

GCM ensemble based climate change prediction

The changes in annual minimum, maximum and mean temperature and precipitation under RCPs 4.5 and 8.5 scenarios for 2030 and 2050 were predicted by ensembling the above 10 GCMs in Diva-GIS 7.5 platform. The data were restricted to the areas where taro is reported to be cultivated as mentioned in an earlier section.

Current and future suitability modelling and suitability change detection

Crop suitability modelling involves the evaluation of the model and the usage of the selected ecological parameter sets to run the model using certain climate scenarios (IPCC 2014). All the suitability analysis were carried out using Diva GIS 7.5 and Arc GIS 10.1 software's. Initially, inputting the calibrated ecological parameters

Table 1. Details of the coupled model inter-comparison project 5 (CMIP5) models selected for the study

Sl. No.	Model	Spatial Resolution (Long ^{0} × Lat $^{0)}$	Modelling Centre	Country
1	CCSM4	1.25 × 0.9424	National Centre for Atmospheric Research	USA
2	CESMI-CAM5	1.25 × 0.9424	National Centre for Atmospheric Research	USA
3	GFDL-CM3	2.5 × 2	NOAA Geophysical Fluid Dynamics Laboratory	USA
4	MIROC-ESM CHEM	2.8125 × 2.7673	Atmosphere and Ocean Research Institute, National Institute for Environmental Studies, Japan Agency for Marine-Earth Science and Technology	Japan
5	NorESMI-M	2.5 × 1.8947	Bjerknes Centre for Climate Research Norwegian Meteorological Institute	Norway
6	INM-CM4	2 × 1.50	Institute for Numerical Mathematics	Russia
7	GFDL-ESM2M	2.5 × 1.5169	NOAA Geophysical Fluid Dynamics Laboratory	USA
8	FIO-ESM	2.815 ×2.7673	First Institute of Oceanography, MNR	China
9	MIROC MIROC 5	1.4063 ×1.389	Atmosphere and Ocean Research Institute, National Institute for Environmental Studies, Japan Agency for Marine-Earth Science and Technology	Japan
10	MPI-ESM-LR	1.25 × 0.9424	Max Planck Institute for Meteorology	Germany

(Table 2) along with the current climate scenario (for current suitability) and for two different future climate scenarios (RCPs 4.5 and 8.5) for the 10 CMIP5 models (for future climate suitability), the suitability of taro was predicted. Table 2 shows that crop would die at a temperature ≤0°C, and is not suited for temperature below 10°C, the crop grows optimally in the range of 21 to 28°C and will not grow if temperature is above 35°C. In the case of precipitation, the crop will be harmfully stressed if the total precipitation during the growing season is less than 1000 mm (drought stress) or above 3000 mm (excess water) and grows optimally in the range of 1500 to 2500 mm precipitation.

Table 2. Ecological parameters used to calibrate the Eco Crop model for taro

Sl. No.	Parameter	Calibrated value
1	Tkill	0°C
2	Tmin	10°C
3	Tmax	35°C
4	Topmin	21°C
5	Topmax	28°C
6	Rmin	1000 mm
7	Ropmin	1500 mm
8	Ropmax	2500 mm
9	Rmax	3000 mm

The suitability change was then calculated for each model and the following impact matrices were derived for taro growing regions for each GCM specific predictions.

a. Average climate suitability change (%) in taro growing regions
b. Average climate suitability change (%) in positively impacted area.
c. Average climate suitability change (%) in negatively impacted area.

The taro presence points were used for extraction of data from the resultant raster maps. Presence points (containing the information of state and district) in the vector format - each placed one square kilometer apart- were laid over the suitability map. Using the tool 'extract value by points' in Diva GIS, the concerned suitability value was extracted. The average value of all the points is accounted as suitability of a district.

Results and Discussion

Projected climate change in taro growing regions

Minimum temperature (T_{min})

The spatial pattern of annual change in minimum temperature at RCPs 4.5 and 8.5 for 2030 and 2050 is shown in Table 3. The T_{min} of major taro growing environments in 2030 is predicted to increase from 1.25 (Tamil Nadu) to 1.65 ^{0}C (Assam) and 1.61 (Kerala) to 2.03°C (Rajasthan) for RCPs 4.5 and 8.5, and the corresponding values for 2050 were 1.37 (Kerala) to 1.74°C (Rajasthan) and 2.16 (Kerala) to 2.83°C (Rajasthan), respectively. For both the scenario and for the years, Kerala and Tamil Nadu showed the lowest increase in T_{min}. The changes in T_{min} by individual GCMs were in the range of 0.41(GFDL-CM3) to 2.04°C (FIO-ESM) for RCP 4.5 and 0.98 (GFDL-CM3) to 2.04°C (MIROC MIROC 5) for RCP 8.5 in 2030; 0.47 (GFDL-CM3) to 2.72°C (FIO-ESM) for RCP 4.5 and 1.81 (GFDL-CM3) to 3.51°C (FIO-ESM) for RCP 8.5, in 2050.

Maximum temperature (T_{max})

T_{max} in 2030 were projected to increase from 0.87 (Meghalaya) to 1.32°C (Rajasthan) and 1.43 (Tamil Nadu) to 2.07°C (Uttar Pradesh) for RCPs 4.5 and 8.5 and the corresponding values for 2050 were 1.17 (Andra Pradesh) to 1.59°C (Uttar Pradesh) and 2.02 (Kerala) to 2.79°C (Uttar Pradesh). The changes in T_{max} by individual GCMs were 0.36 (CESMICAM5)

Table 3. Predicted climate change in major taro growing regions of India

	Change in minimum temperature (°C)				Change in maximum temperature (°C)				Change in mean temperature (°C)				Change in precipitation (mm)			
RCP	4.5		8.5		4.5		8.5		4.5		8.5		4.5		8.5	
Year	2030	2050	2030	2050	2030	2050	2030	2050	2030	2050	2030	2050	2030	2050	2030	2050
Andhra Pradesh	1.30	1.71	1.43	2.37	1.01	1.62	1.17	2.21	1.21	1.73	1.35	2.17	22.22	42.81	13.51	61.71
Assam	1.65	1.95	1.63	2.68	0.87	1.51	1.31	2.35	1.31	1.78	1.52	2.43	82.07	128.02	15.19	108.54
Bihar	1.42	1.76	1.56	2.67	1.10	1.75	1.38	2.55	1.30	1.82	1.52	2.47	19.06	22.10	15.19	52.67
Kerala	1.31	1.61	1.37	2.16	0.93	1.44	1.26	2.02	1.18	1.59	1.36	2.02	24.86	7.37	13.60	1.09
Meghalaya	1.47	1.81	1.54	2.59	0.87	1.51	1.21	2.32	1.22	1.72	1.43	2.32	69.50	77.90	14.26	9.29
Rajasthan	1.56	2.03	1.74	2.83	1.32	2.02	1.55	2.62	1.49	2.09	1.70	2.68	15.71	14.67	16.98	90.23
Tamil Nadu	1.25	1.62	1.38	2.20	0.94	1.43	1.21	2.11	1.15	1.58	1.35	2.05	38.06	57.72	13.48	91.22
Telangana	1.49	1.90	1.60	2.70	1.18	1.88	1.38	2.56	1.40	1.97	1.54	2.41	9.08	41.55	15.38	97.99
Uttar Pradesh	1.49	1.89	1.67	2.81	1.32	2.07	1.59	2.79	1.45	2.04	1.68	2.68	-2.01	2.84	16.80	49.29
West Bengal	1.36	1.67	1.41	2.52	1.11	1.73	1.27	2.48	1.29	1.77	1.39	2.28	35.14	28.34	13.90	56.04

to 1.671°C (FIO-ESM) for RCP 4.5 and 1.06 (GFDL-CM3) to 1.925°C (MIROC-ESM CHEM) for RCP 8.5 by 2030. The changes in 2050 for RCP 4.5 were from 1.15 (MPI-ESM-LR) to 2.49°C (FIO-ESM) and for RCP 8.5 the corresponding values were 1.63 (NorESMI-M) - 3.76°C (FIO-ESM).

Mean temperature (T_{mean})

The T_{mean} of major taro growing environments in 2030 were projected to increase by 1.15 (Tamil Nadu) - 1.49°C (Rajasthan) and 1.58 (Tamil Nadu) – 2.09°C (Rajasthan) for RCPs 4.5 and 8.5 and the corresponding values for 2050 were 1.35 (Tamil Nadu and Andra Pradesh) to 1.70°C (Rajasthan) and 2.02 (Kerala) to 2.68°C (Rajasthan and Uttar Pradesh), respectively. The individual GCM predicted changes in Tmean were in the range of 0.70 (GFDL-CM3) - 1.91°C (FIO-ESM) and 1.07 (GFDL-CM3) – 2.04°C (MIROC MIROC 5) for RCPs 4.5 and 8.5 in 2030. The corresponding values in 2050 ranged from 1.02 (GFDL-CM3) to 2.65°C (FIO-ESM) and 1.73 (GFDL-CM3) to 3.53°C (FIO-ESM).

Annual rainfall

In 2030, the rainfall would change from -2.01 (Uttar Pradesh) to 82.07 mm (Assam) and 2.84 (Uttar Pradesh) to 128.02 mm (Assam) for RCPs 4.5 and 8.5. The corresponding values for 2050 were 13.48 (Tamil Nadu) to 16.98 mm (Rajasthan) and 1.09 mm (Kerala) to 108.54 mm (Assam). The individual GCM predicted changes in rainfall varied from -26.6 (MIROC-ESM CHEM) to 102.66 mm (NorESMI-M) for RCP 4.5 and -22.2 (GFDL-CM3) to 99.68 mm (GFDL-ESM2M) for RCP 8.5 in 2030. In 2050, it would be from -99.7 (MPI-ESM-LR) to 159.59 mm (NorESMI-M) for RCP 4.5 and 1.00 (MIROC MIROC 5) to 217.51 mm (CEMI-CAM5) for 8.5.

Current climate suitability of taro

With the calibrated ecological parameters, the current climate suitability of taro was modelled using Ecocrop model in DIVA-GIS 7.5 (Fig. 2). The results showed an average suitability between 10.75 and 85.95% in major taro growing regions. Kerala (85.95%) showed excellent suitability (>80%) whereas Assam (77.65%) and West Bengal (66.92%) were very suitable for taro cultivation, while Meghalaya and Bihar showed suitability of 56.23 and 51.02%, respectively.

Future climate suitability of taro

Predicted future suitability of taro in major growing regions of India is shown in Fig. 3. In 2030, the result showed an average future suitability between 12.66 and 76.34% in major taro growing regions; it is predicted to be 76.34% for Kerala, 72.64% for Assam, 61.38% for Meghalaya, 54.60% for West Bengal and rest of states shows future suitability below 50% for RCP 4.5. For

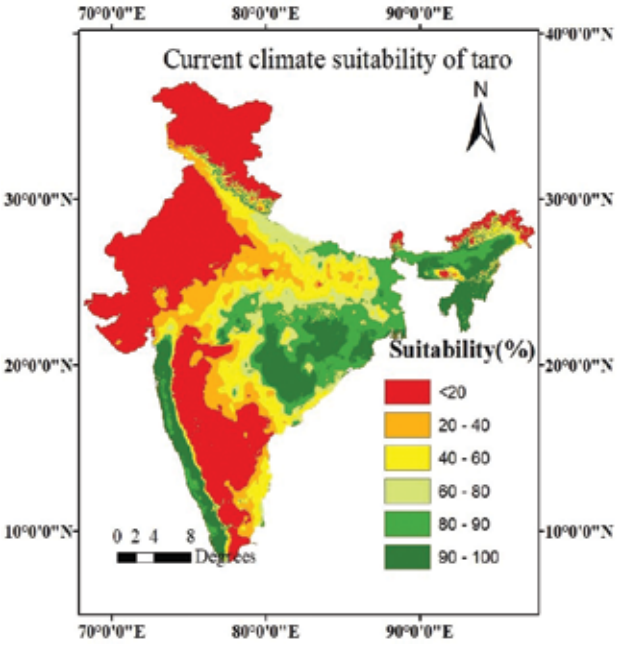

Fig. 2. Current climate suitability of taro by EcoCrop model

RCP 8.5, the predicted values would range between 13.74 and 73.69%, and it would be 73.69% for Kerala, 72.52% for Assam, 63.85% for Meghalaya, 52.64% for West Bengal and rest of states shows future suitability below 50% for both the RCPs. For RCP 4.5 in 2050, the future suitability was in the range 12.82 to 72.83% and it will be 72.83% for Kerala, 69.59% for Assam, 62.79% for Meghalaya, 95.04%. For RCP 8.5, the suitability's would range between 16.70 and 69.51%, which will be 69.51% for Kerala, 66.48% for Assam, 65.61% for Meghalaya and all other states show future suitability below 50% for both RCPs.

The changes in current suitability of taro under RCPs 4.5 and 8.5 by 2030 and 2050 are shown in Fig. 4 and their impacts in Table 4. The model ensembled results under RCP 4.5 in 2030 ranged from -12.31 (West Bengal) to 5.17% (Meghalaya). Decrease in suitability was observed for West Bengal (-12.31%), Kerala (-9.61%), Assam (-5.02%), Bihar (-3.5%) and Uttar Pradesh (-2.38%) and Andra Pradesh (0.4%), Telangana (0.47%), Tamil Nadu (1.5%), Rajasthan (1.92%) and Meghalaya (5.17%) showed a suitability increase. Individual GCM predicted changes in taro growing regions ranged from -7.91 (MIROC-ESM CHEM) to 5.13% (NorESMI-M). Under RCP 8.5 in 2030, the decrease in future suitability was observed in West Bengal (-14.29%), Kerala (-12.27%), Assam (-5.13%), Uttar Pradesh (-3.03) and Bihar (-2.91%). Rajasthan, Tamil Nadu, Andhra Pradesh, Telangana and Meghalaya showed increased suitabilities of 2.98, 3.38, 3.81, 3.97 and 7.63% respectively. Studies with individual GCMs showed variabilities from -5.74 (GFDL-CM3) to 2.94% (NorESMI-M) for RCP 8.5, 2030.

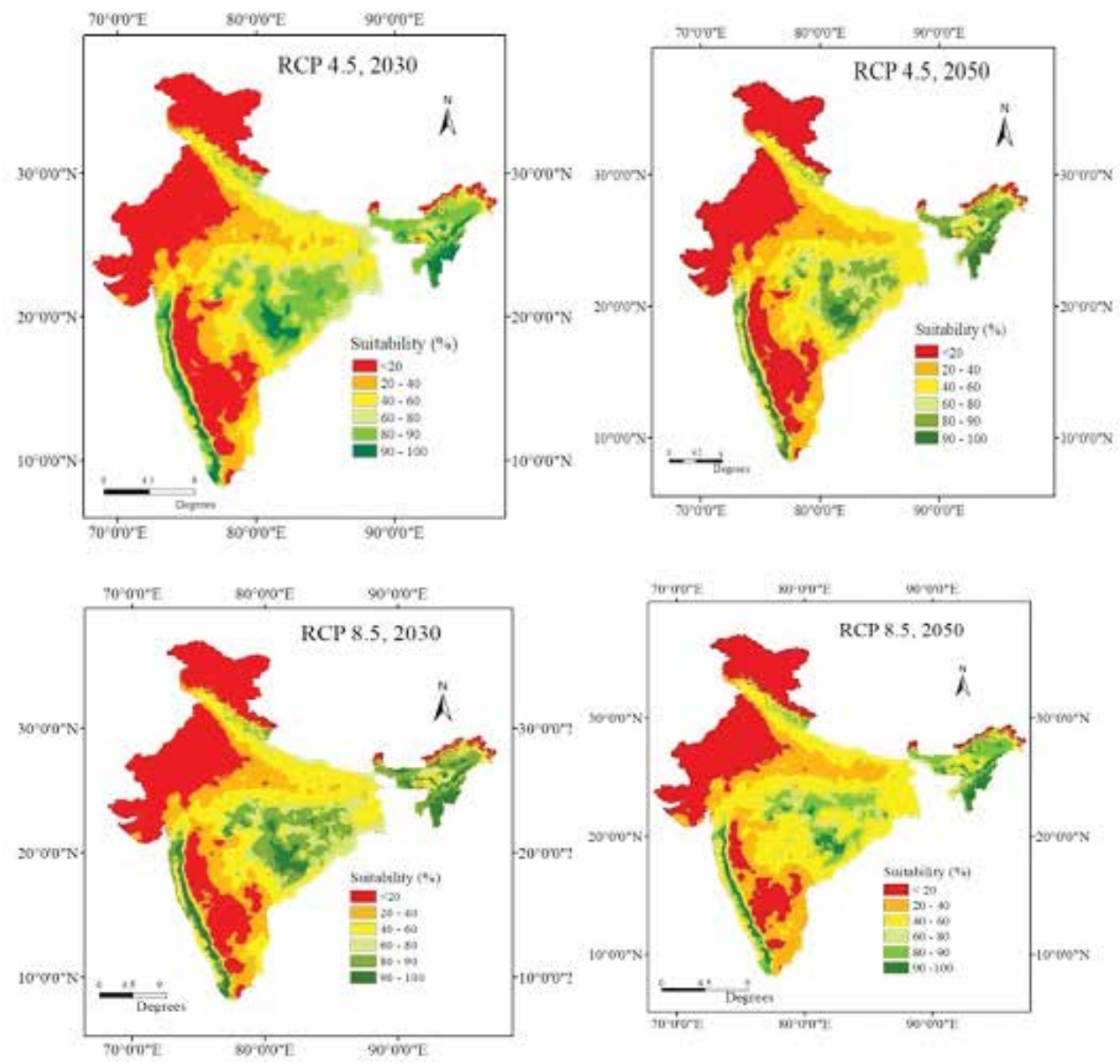

Fig. 3. Predicted future suitabilities of taro as average of the 10 GCMs studied

Change in climate suitability and impacts on taro at different scenarios

The predicted changes in climate suitability in districts of major taro growing states – Andhra Pradesh, Assam, Kerala, Meghalaya, Tamil Nadu and Uttar Pradesh have showed that, for RCP 4.5 in 2030, Vishakhapatnam (5.60%) in Andhra Pradesh, Tinsukia (14.70%) in Assam, Kozhikode (11%) in Kerala, West Khasi Hills (15%) in Meghalaya, Tirunelveli (9.70%) in Tamil Nadu and Sharanpur (13.90%) in Uttar Pradesh showed highest positive impact. While Chittoor (-12.90%), N.C. Hills (-17.20%), Kannur (-21.30%), West Garo Hills (-15.70%), Vellore (-7.60%) and Mau (-13.7%) in Andhra Pradesh, Assam, Kerala, Meghalaya, Tamil Nadu and Uttar Pradesh, respectively showed highest negative impact. For RCP 8.5 in 2030 Chittoor (10.90%), Chirang (17.40%), Kozhikode (13.5%), West Khasi Hills (19%), Tirunelveli (11.40%) and Bunor (17%) in Andhra Pradesh, Assam, Kerala, Meghalaya, Tamil Nadu and Uttar Pradesh showed highest positive impact; while West Godavari (-14.10%) in Andhra Pradesh, Karbi Analog (-20%) in Assam, Malappuram (-26.40%) in Kerala, Ri Bhoi (-18.40%) in Meghalaya, Vellore (-9.60%) in Tamil Nadu and Sharanpur (-23%) in Uttar Pradesh showed highest negative impact.

For RCP 4.5 in 2050, individual GCM predicted changes would be from -13.49 (MPI-ESM-LR) to 4.96% (NorESMI-M). Model ensembled results for the same showed a decrease in future suitability in West Bengal (-18.26%), Kerala (-13.13%), Assam (-8.07%), Bihar (-7.37%) and Uttar Pradesh (-5.01%). Andhra Pradesh,

Table 4. Regional changes in taro climate suitability for individual GCMs studied

GCM	RCP 4.5, Year 2030			RCP 8.5, Year 2030			RCP 4.5, Year 2050			RCP 8.5, Year 2050		
	OSC*	SCPIA*	SCNIA*	OSC	SCPIA	SCNIA	OSC	SCPIA	SCNIA	OSC	SCPIA	SCNIA
CCSM4	1.25	7.16	-10.87	-0.72	7.96	-9.23	-1.18	7.61	-11.03	-6.79	8.97	-14.67
CESMI-CAM5	-2.50	7.58	-6.29	0.57	6.27	-7.46	-3.89	8.05	-9.88	-2.34	20.82	-13.50
GFDL-CM3	-4.92	6.28	-10.62	-5.76	9.55	-10.81	-7.65	7.74	-16.36	-4.61	15.69	-11.92
MIROC ESM-CHEM	-3.19	4.87	-5.29	-5.67	9.50	-10.18	-6.47	3.92	-9.17	-6.27	8.88	-12.60
NorESMI-M	-1.76	6.10	-7.79	-1.25	6.32	-8.43	0.89	9.58	-13.43	-3.60	9.76	16.22
INM-CM4	-2.17	6.14	-6.88	-1.65	5.75	-7.44	-0.26	7.37	-9.54	-3.90	7.63	12.61
GFDL-ESM2M	-2.97	3.92	-7.42	0.05	9.43	-8.48	-8.88	6.17	-12.55	-12.95	8.06	-17.99
FIO-ESM	-7.94	4.77	-11.22	-4.92	6.68	-9.40	-7.77	6.12	-11.82	-12.42	10.61	-17.75
MIROC MIROC 5	-5.15	3.50	-7.00	-2.69	5.08	-9.56	-13.54	5.57	-16.27	-6.99	5.55	-14.63
MPI-ESM-LR	5.15	9.15	-6.81	2.95	10.52	-12.51	4.98	10.49	-9.26	4.03	11.85	12.10
Mean	-2.42	5.95	-8.02	-1.91	7.706	-9.35	-4.38	7.26	-11.93	-5.58	10.78	-6.21

OSC* - Overall suitability change, SCPIA - Suitability change in positively impacted areas, SCNIA - Suitability change in negatively impacted area.

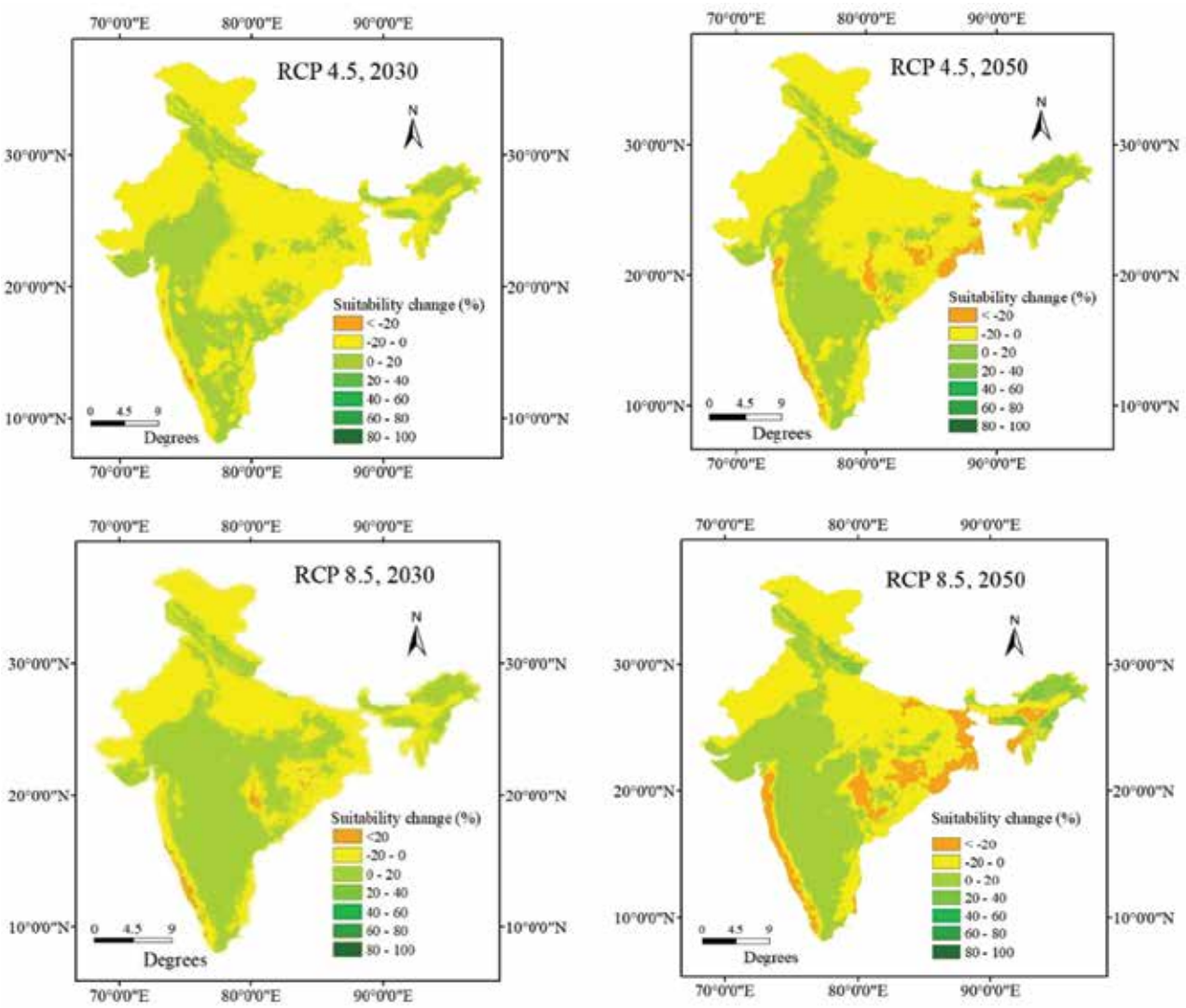

Fig. 4. Predicted changes in taro climate suitabilities as average of the 10 GCMs studied

Tamil Nadu, Rajasthan, Telangana and Meghalaya showed increased suitabilities of 0.85, 1.41, 2.08, 2.48 and 6.57% respectively. For RCP 8.5 in 2050, the individual GCMs showed variability from -12.9 (MIROC MIROC 5) to 4.02 % (NorESMI-M). West Bengal (-24.1%), Kerala (-16.45%), Bihar (-12.5%), Assam (-11.17%) and Uttar Pradesh (-6.01%) showed decrease in suitability, whereas Andhra Pradesh (1.66%), Tamil Nadu (2.01%), Telangana (3.73), Rajasthan (5.95%) and Meghalaya (9.39%) showed an increase of suitability for taro.

In the districts of major taro growing states for RCP 4.5 in 2050, Chittor (7.2%) in Andra Pradesh, Tinsukia (19.70%) in Assam, Malappuram (15.30%) in Kerala, West Khasi Hill (19.9%) in Meghalaya, Tirunelveli (11.9%) in Tamil Nadu and Sharanpur (18.5%) in Uttar Pradesh showed highest positive impact, while West Godavari (18.6%), Karbi Analog (-22.30%), Ernakulam (-25%), West Garo Hills (-22.5%), Vellore (-13.5%) and Mau (-18.9%) in Andhra Pradesh, Assam, Kerala, Meghalaya, Tamil Nadu and Uttar Pradesh showed highest negative impact. For RCP 8.5 in 2050, Chittoor (12.6%) in Andra Pradesh, Karbi Analog (28.60%) in Assam, Wayanad (18.80%) in Kerala, East Khasi Hills (33.40%) in Meghalaya, Tirunelveli (18.70%) in Tamil Nadu and Sharanpur (21.90%) in Uttar Pradesh showed highest positive impact in taro suitability while West Godavari (-28.50%) in Andra Pradesh, Dhubri (-28.30%) in Assam, Kannur (-32.30%) in Kerala, West Garo Hills (33.40%) in Meghalaya, Vellore (-17.4%) in Tamil Nadu and Deoria (-24.0%) in Uttar Pradesh showed highest negative impact. Overall suitability change (OSC) is predicted to be negative for both the years and scenarios, except for CCSM4 (1.25%) and MPI-ESM-LR (5.15 %) for 4.5 scenario and 0.57, 0.05 and 2.95% for CESMI-CAM5, GFDL-ESM2M AND MPI-ESM-LR, respectively for 8.5 scenario in the year 2030. In 2050, the OSC for models Nor ESMI-M (0.89%) and MPI-ESM-LR (4.98%) in 4.5 scenario and in 8.5, MPI-ESM-LR (4.03%) were predicted to be positive. Positively impacted areas would be more for RCP 8.5 in 2030 and minimum area would have positive impact for RCP 8.5 in 2050. For both RCPs of 4.5 and 8.5 in 2030 and 2050, the result showed that the positive impact would be less. Warming of the atmosphere, mere increase in total rainfall and climate suitability of taro are predicted under RCPs 4.5 and 8.5 in 2030 and 2050 (Fig. 5). Kodis et al., (2018) studied ecological niche modeling for a cultivated plant species: a case study on taro (*Colocasia esculenta*) in Hawaii using two ecological niche models. The findings also imply that climate change will have an impact on the geographic regions that are projected to be suitable for taro, with more extreme future climatic scenarios showing less overlap between these regions and

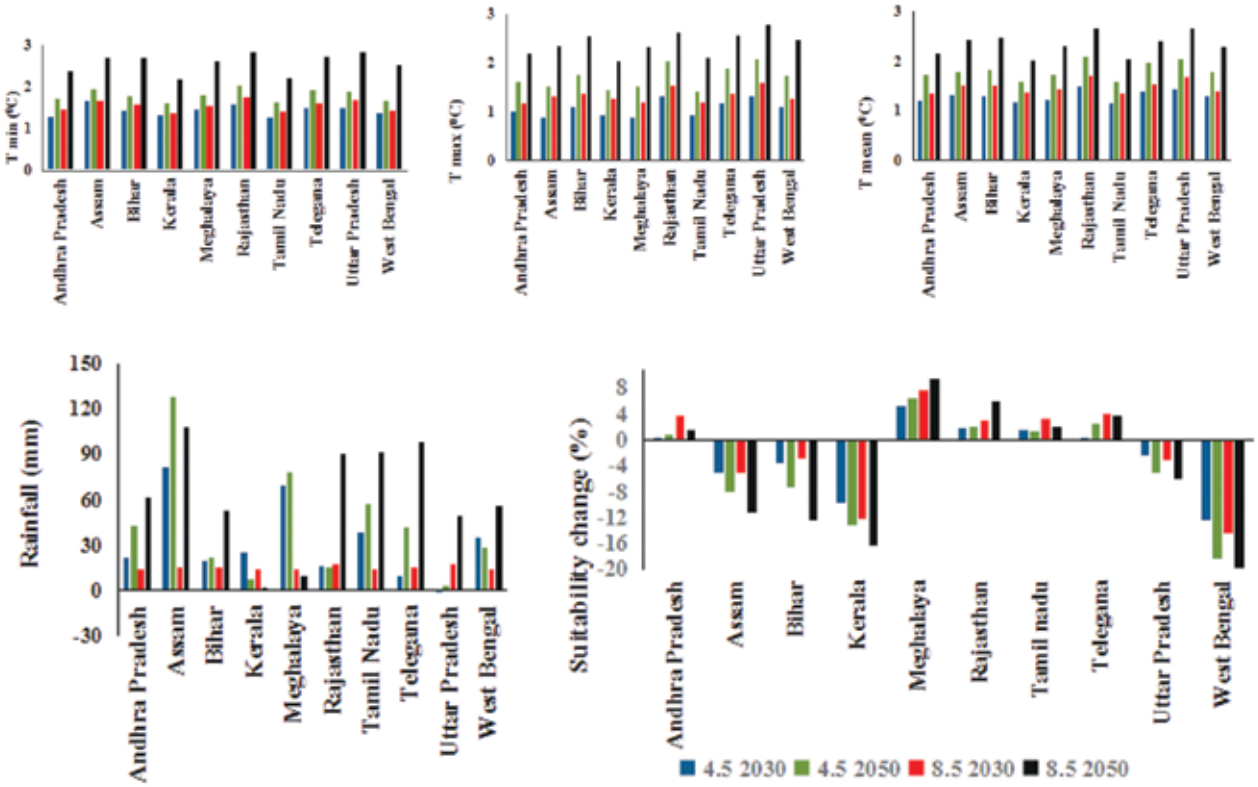

Fig. 5. Comparison of climate change and climate suitability change in the taro growing regions under RCP for 2030 and 2050

existing habitat. Pushpalatha et al., (2023) studied the future climate suitability of underutilized tropical tuber crops by using MaxEnt model, suggested that taro was highly suitable in southern peninsular and north- eastern regions in near future (2030) which was in accordance with our result.

Conclusion

According to the results of the current study, 2050 under the RCP 8.5 scenario would be the warmest and 2030 under any scenario were anticipated to be warmer than the current climate. No accountable changes in precipitation were predicted by both scenarios for both time periods. The mean temperature of major taro growing regions in 2030 will increase by 1.15- 1.49°C and 1.58 – 2.09°C for RCPs 4.5 and 8.5; and 1.35 - 1.70°C and 2.02 - 2.68°C for RCPs 4.5 and 8.5 in 2050. The precipitation in 2030 will increase by -2.01 – 82.07 mm and 2.84 - 128.02 mm for RCPs 4.5 and 8.5; and in 2050 it will change by 13.48 to 16.98 mm and 1.09 to 108.54 mm for RCPs 4.5 and 8.5. The climate suitability will change by -12.31 to 5.17% and -14.29 to 7.63% in 2030 for RCPs 4.5 and 8.5; and -18.26 to 6.57% and -24.1 to 9.39% for RCPs 4.5 and 8.5 in 2050. The suitability of taro under RCPs 4.5 and 8.5 by 2030 and 2050 indicated that there would be a significant change in suitability. The individual GCMs predicted results at two different scenarios for the two time periods, showing that though there were positively impacted areas, the overall suitability was negative, or predicted to decrease by most of the GCMs.

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SNP marker development in cassava for cassava mosaic disease resistance using bioinformatics tools

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Abstract

Cassava (*Manihot esculenta* Crantz), originated in Latin America is one of the most important food crops with a worldwide production of 314.81 million tonnes. The advancements in sequencing ability and less cost involved allow for effective genome-wide discovery of SNPs. The present study was undertaken to computationally develop SNPs for cassava mosaic disease resistance and to understand the effectiveness of molecular markers in cassava for biotic stress response (cassava mosaic virus). The preliminary data set for the work was obtained from the EST section of NCBI (http://www.ncbi.nlm.nih.gov/nucest). The draft cassava genome sequence and transcript sequences (variety AM560-2, JGI annotation v4.1) from the Phytozome website (http://phytozome.jgi.doe.gov/pz/portal.html) were also utilized. The SNP prediction tools, viz., Quality SNP and Auto SNP were used to predictthe SNPs. Quality SNP predicted about 56 SNPs, in which 30 were non-synonymous and 26 were synonymous SNPs. Primers were designed for five selected SNPs associated with CMD resistant genes. These primers were validated using 5 resistant and 5 susceptible cassava genotypes. Among the primers, after validation one SNP (SNP896) primer was able to clearly differentiate between the resistant and susceptible genotypes. This is the first report of SNPs computationally identified and verified in wet lab. The results showed that the sequence with SNP1043 did not show any variation in the predicted SNP site, but SNP896 in the variety, MNga showed SNP at the 1493th position with a variation in the base. The same SNP896 did not show any variance in that position for the susceptible variety CI732.

Keywords: Cassava mosaic disease, SNP marker, Bioinformatics, Molecular markers

Introduction

Cassava, (*Manihot esculenta* Crantz) (2n = 36), which originated in Latin America is an important food crop with a worldwide production of 314.81 million tonnes, with the highest production of 203.57 million tonnes in Africa, followed by Asia (84.25 million tonnes)(FAOSTAT 2022). Cassava is an essential staple food for over 700 million people all over the tropical and sub-tropical regions of the world. It can be grown all year round and provides food in periods of scarcity. Various traits of the crop such as drought tolerance, heat tolerance and less requirement for agricultural fertilizers make it an attractive crop. Cassava has monoecious flowering nature and so self-pollination is mainly prevented by protogynywhich renders the crop highly heterozygous (Alves, 2002). The high starch content (20-40%) makes cassava a desirable energy source both for human consumption and industrial biofuel applications (Schmitz et al., 2009). Cassava is one of the most used raw materials to produce starch. High purity, low production costs, distinctive characteristics like clear viscous paste has made many industries adopt cassava starch as an alternative to more traditional sources like potato and maize.

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Cassava mosaic disease (CMD) is the single most important disease affecting cassava cultivation. Economic losses due to CMD is estimated at US $1.5 billion annually in Africa (Legg et al.,2006; Thresh et al., 1997). CMD is caused by gemini viruses of the genus Begomovirus (Family Geminiviridae) transmitted by a vector, white fly [*Bemisia tabaci, (Gennadius)*]. The begomoviruses represent distinct species such as African cassava mosaic virus (ACMV), East African cassava mosaic virus (EACMV), East African cassava mosaic Cameroon virus, East African cassava mosaic Zanzibar virus and South African cassava mosaic virus (Berrie et al., 2001). The causative agent of CMD in India is Indian cassava mosaic virus, ICMV (Hong et al., 1993). Complete nucleotide sequencing of two cloned ICMV DNAs, one from the state of Kerala and another from Maharashtra showed that they were highly like each other, indicating them to be isolates of the same virus (Saunders et al., 2002).

Availability of Nucleotide sequence information of cassava has effectively helped in the discovery of several genes including disease resistant genes. Expressed sequence tags (ESTs), which are short (300–500 bp) single read sequences from random cDNA clones, have a wide range of applications including the use as gene cloning reservoirs, evaluation of expression of tissue-specific gene, molecular markers for map based cloning and genomic sequence annotation. The EST data have also led to a better understanding of both the existence and expression patterns of alternative transcripts and of coordinated gene expression and itrepresents a potentially significant resource for the detection of single nucleotide polymorphism (SNPs) in plants (Batley et al., 2003). Analysis of ESTs has advanced into an economical and capable gene discovery methodology (Ohlrogge et al., 2003). About 74,316,793 million ESTs are available at the EST database of National Center for Biotechnology Information (NCBI). The genome of cassava is approximately 770 Mb (Awoleye et al., 1994), and the draft genome sequence of cassava was created through the whole genome shotgun strategy. The whole genome of cassava is available in Phytozome, which was developed as part of the global cassava partnership in the year 2003. The cassava genome is predicted to contain 30,666 genes (Prochnik et al., 2012). However, the function of many of the genes remains unclear.

Plant genetic and physical mapping resources along with breeding programes in different agricultural crops led to the development of various plant databases like *Brassica.info, PlantGDB(13), Plaza(14), Ensemblgenomes(16), GSAD(17), NCBI, PGDJ, Phytozome, TAIR, Plant DNA C-values database, Gramene(15), Plant rDNA Database, SGN, The Plant GDB Genome Browser, GDR, LIS, and PTG Base*. These databases consist of genomic data which can be downloaded and used for further analysis and they provide a set of automated analysis tools within the web portals.

In plant genetic research, molecular markers are used for effective marker assisted selection, population structure analysis, evolutionary relationship study and whole genome studies. Molecular markers such as Single Nucleotide Polymorphisms (SNPs) and Single sequence repeats (SSRs) has high potential to help plant breeders. SNPs are markers of choice for high-density genetic mapping due to their sheer abundance in the genome (Rafalski, 2002). SNPs are known to occur at a rate of one per 100-500 bp in plant genomes, depending on the species. The advancements in sequencing ability along with the savings in sequencing cost allow for effective genome-wide discovery of SNPs. RNA-seq has been successfully applied to large-scale SNP discovery and EST- derived SNP development in various plant species (Ferguson et al., 2012; Paritosh et al., 2013). The present study was aimed at developing computationally predicted SNP makers for cassava mosaic disease resistance. The SNP development tools were also evaluated to understand their performance.

Materials and Methods

The detailed workflow used for the SNP prediction is given in Fig. 1.

Primary cassava dataset

The preliminary data set which consists of a total of 86310 ESTs was obtained from the EST section of NCBI (http://www.ncbi.nlm.nih.gov/nucest) and the transcript sequences consisting of 34151 sequences of cassava (variety AM560-2, JGI annotation v4.1) was downloaded from the Phytozome website (http://phytozome.jgi.doe.gov/pz/portal.html). Together, a total of 1,20,461 sequences were taken as the primary dataset. The sequences were pre-processed using the Seq Clean script (http://sourceforge.net/projects/seqclean/files/) with the default runtime options for eliminating contamination or simple repeats. Vector sequences in these ESTs were then trimmed using the UniVec_Core database (http://www.ncbi.nlm.nih.gov/tools/vecscreen/univec/) of NCBI. Sequences with more than 96 percentage similarity to a contaminant was removed. Based on cultivars, the sequences were classified into 19 categories and one category with unclassified sequences. All the phytozome transcript sequences used were of a cassava cultivar named Am560-2 with 34151 sequences. Most sequences in NCBI were from MTai-16 with 35400 sequences and sequences with the least number was of the cultivar H-226 which had only 21 sequences.

Virus resistant gene database

Virus resistant gene database including CMD resistant gene database was manually created and compiled from uniprot KB. The UniProt Knowledgebase (UniProt KB) is the central access point for extensively curated protein information, including function, classification,

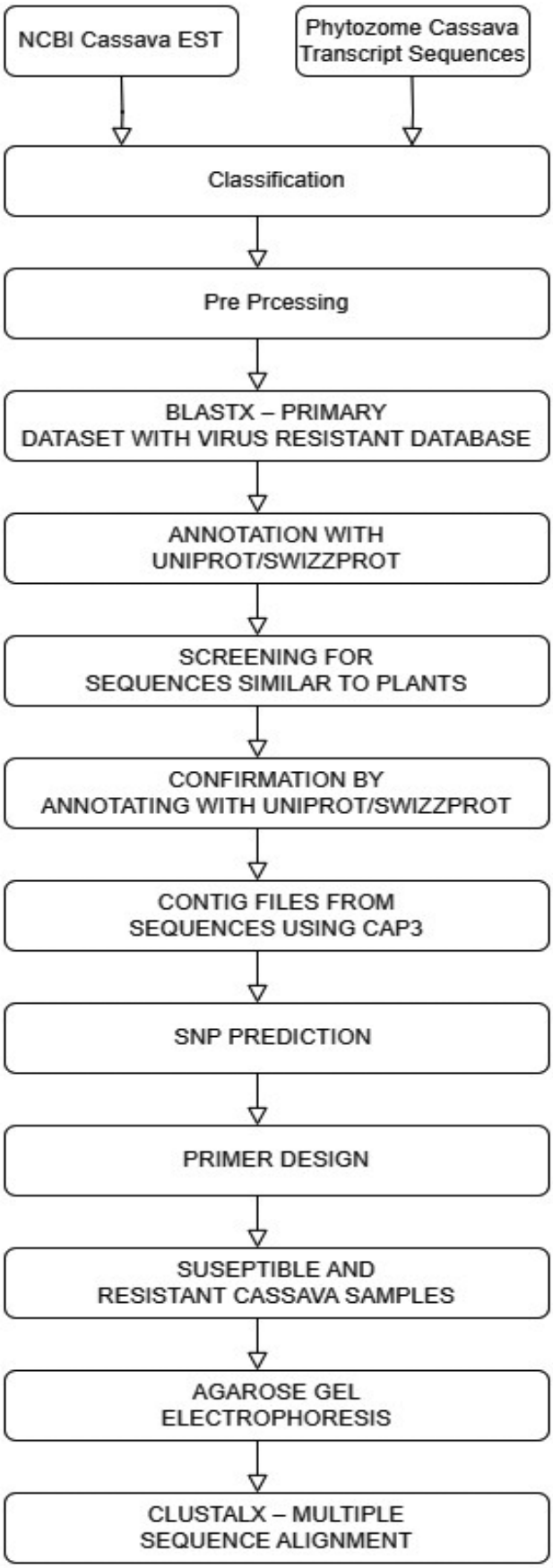

Fig. 1. Workflow for the prediction and validation of SNP

and cross-references. R-gene or resistant genes related to cassava and mosaic diseases was screened from it and were used for database creation. The virus resistance protein database consisted of 730 resistant genes.

Processing of primary dataset

For screening of primary dataset with virus resistant protein sequences, 'BlastX- Search protein database using a translated nucleotide query' was employed. Klast was used for doing sequence comparison (http://koriscale.inria.fr/). The cassava ESTs and transcript sequences were screened against resistant genes using BLASTX. Resulting cassava ESTs and transcript sequences were annotated using Uniprot/SwissProt database and only sequences which have functional annotations were retained. Nucleotide sequenceswith similarity to organisms other than plants were eliminated. The resulting screened sequence datasetwas used for DNA polymorphism discovery.

DNA polymorphism studies

Assembling of the screened sequence datasetof sequences was carried out using the Perl script CAP3 program (http://seq.cs.iastate.edu/CAP3.html) with default runtime options. SNP and InDelpolymorphisms were discovered from the contigs obtained. Quality SNP pipelinewas used for the discovery of SNP and InDels (http://www.bioinformatics.nl/tools/snpweb/). Analysis of the alignment information to select cluster size of four was done using the Perl script 'Getalignmentinfo'. The Cprograms 'Getavailcontigseq' and 'Getavailcontigqual' extracted the sequences from the contigs and delivered the quality information of contigs. Using C program 'QualitySNP' predicted SNPs and InDels. Another C program named 'Getnonsy SNP fasty' was used to analyze the FASTY results, detect the ORFs and find non-synonymous SNPs. For the analysis of non-synonymous SNPs, Viridiplantae database was used(tp://141.161.180.197/pub/databases/uniprot/current_release/knowledgebase/taxonomic_divisions/uniprot_sprot_plants.dat.gz).

Verification in wet lab

Primer designing for predicted SNPs

Primer pairs were designed to amplify the genomic region around each discovered SNP site. Sequences were selected for primer designing based on the hit percentage of contigs containing SNP with the resistant genes. SNP containing contigs with a hit percentage between 80-100% were selected. Primer pairs were designed using Primer3plus tool with the parameters set as (i) GC content above 50% and (ii) Melting temperature between 55 and 60°C.

Plant materials

A total of 10 cassava varieties which included 5 CMD resistant and 5 susceptible varieties were selected based

on field trials conducted at ICAR-Central Tuber Crop Research Institute (CTCRI), Thiruvananthapuram. Fresh young leaves were collected, and DNA was isolated from these leaf samples using the method described by Dellaporta et al., (1983) with some modifications. The concentration and purity of all the DNA samples was determined using a UV spectrophotometer by taking absorbance at 260 nm. The amount of DNA was quantified using the following formula:

$$\text{DNA concentration } (\mu g\ ml^{-1}) = \frac{OD260 \times 100 \text{ (dilution factor)} \times 50}{1000}$$

According to the reading obtained after quantification, genomic DNA was diluted to a concentration of 50 ng μl^{-1} and stored at 4°C. The stock DNA was then stored in -20°C.

Amplification of designed primers

Primer amplification was done in a BioRad C1000™ thermal Cycler with respective parameters for SNP and SSR primers.

Validation of SNP markers

Validation of SNP markers was done by running the marker with the DNA isolated from the five resistant and five susceptible cassava varieties in agarose gel electrophoresis and then eluting the bands, sequencing it, and comparing it with the reference genome of cassava. Reference genome of cassava is available in the Phytozome database. ClustalX was used for aligning the sequences and to validate the SNP.

Results and Discussion

Cassava dataset and screening process

The preliminary data consisted of 86310 sequences from the EST section of NCBI and 34151 transcript sequences from the Phytozome database. From 19 cassava cultivars (arg7, Cas 36.04, Cm 523-7, cm 21772, crantz, Iac 12.829, ku 50, mper183, mbra 685, mcol 1522, h 226, mirassol, mnga 2, mtai 16, 'Sauti, Gomani, Mbundumali, TME1 and Mkondezi', g 107-35, w 14, Cas 36.01 and Am 560-2), 97921 sequences and from unknown varieties, 22540 sequences were obtained. The sequences were cleaned for contaminants and a total of 120398 sequences were obtained as the starting data for the study. The primary dataset was screened against virus resistance gene database using BLASTX and 16299 sequences were obtained with similarity to R-genes. About 86% *i.e.*, 104099 sequences were screened out by this process.The sequence with similarity to R-genes were annotated using Uniprot/Swissprot database and the number of sequences after annotation was 15796 and about 3% *i.e.*, 503 sequences were screened out. About 1460 sequences were eliminated because of the presence of sequence similarity to organisms other than plants.

DNA polymorphism study

The sequences after screening were aligned and assembled using cap 3. 2088 contigs and 5236 singlets were obtained from 14336 sequences with similarity to virus resistant genes. Quality SNP was able to identify 128 SNPs. From 2088 contigs, a total of 3297227 sequences were examined. In this study, about 204 SNPs were predicted which are exclusievely related to CMD resistance in cassava. These can be validated and screened for effective markers against CMD resistance. More than 56 SNPs were confirmed in the coding region which makes them candidate SNPs for screening for resistance against CMD. More than 30 SNPs were nonsynonymous which can result in change in the transcription product.

A total of 121 SNPs were identified using Quality SNP. The total number of transitions (67) was marginally greater than the total number of transversions (54) yielding a transition-to-transversion ratio of 1.24 (Table 1). Based on the annotation data, these SNPs were classified as SNPs in coding region, non-coding region and in untranslated region. About 56 SNPs were found in the coding region and these can alter proteins. About 65 SNPs were predicted from non-coding region and 76 from untranslated region. Based on the type of SNPs, these were further classified into synonymous SNPs and nonsynonymous SNPs. About 30 SNPs were nonsynonymous SNPs (Table 2), which means that they will effect a change in the translated protein. About 26 SNPs were synonymous (Table 3), *i.e.*, the mutations will not cause any change in the system. Again, based on the type of polymorphism these SNPS can be classified into SNPs and InDels. About 72 SNPs and 56 InDels are obtained.

Table 1. Distribution of transition and transversion SNPs from QualitySNP

Characterization	Type of SNP	SNPs	Total
Transition	C/T	33	67
	G/A	34	
Transversion	A/C	14	54
	A/T	11	
	C/G	17	
	T/G	12	

Quality SNP showed more promising SNPs than Auto SNP, where a huge number of SNPs including false positive SNPs were also predicted. Quality SNP showed unique ability to annotate and classify SNPs based on their polymorphism, the type of annotation data and the type of SNP. However, in Auto SNP, classification was entirely based on the type of SNPs. Quality SNP gave a more detailed and precise information whereas Auto SNP predicted thousands of SNPs with difficulty in identifying the viable ones from the enormous list of identified SNPs (Table 4).

Table 2. List of Nonsynonymous SNP coding data identified by QualitySNP

Contig no.	Position	SNP	Length	Normal sequence	Sequence with base change	Transcribed protein	
260	388	TC	10	CACCAGAATTTATCATCAAGC	CACCAGAATTCATCATCAAGC	HQNLSSS	HQNSSSS
344	509	AT	10	AAATCAGCTTATGCATTGTGT	AAATCAGCTTTTGCATTGTGT	KSAYALC	KSAFALC
385	683	GC	10	AACAGTGAGAGCAAACAAGAG	AACAGTGAGACCAAACAAGAG	NSESKQE	NSETKQE
401	630	GT	11	TTGCGCAAGCAGTACGGACCT	TTGCGCAAGCATTACGGACCT	LRKQYGP	LRKHYGP
401	833	GC	10	CGGAATCCAAGGAAAAGGCTA	CGGAATCCAACGAAAAGGCTA	RNPRKRL	RNPTKRL
401	836	GA	10	AATCCAACGAGAAGGCTATCA	AATCCAACGAAAAGGCTATCA	NPTRRLS	NPTKRLS
468	1143	AG	9	GCTGCATTCAATATGCCACCC	GCTGCATTCGATATGCCACCC	AAFNMPP	AAFDMPP
732	82	CA	11	GTTCAATCTCACCCCAGAAGC	GTTCAATCTCAACCCAGAAGC	VQSHPRS	VQSQPRS
896	1495	CA	10	GTGCTATATACGCACCCAGCA	GTGCTATATAAGCACCCAGCA	VLYTHPA	VLYKHPA
1043	635	CT	10	TCTCAAACAACGATTTATGTG	TCTCAAACAATGATTTATGTG	SQTTIYV	SQTMIYV
1053	1044	TG	11	TGTCAGGGAGATTATGTGGTG	TGTCAGGGAGAGTATGTGGTG	CQGDYVV	CQGEYVV
1073	76	CT	10	CGTGAACAACCTCCCTCCATC	CGTGAACAACTTCCCTCCATC	REQPPSI	REQLPSI
1073	79	TC	10	GAACAACCTCTCTCCATCCTC	GAACAACCTCCCTCCATCCTC	EQPLSIL	EQPPSIL
1073	126	TC	9	TTTGGCTCTTTTTCTCCCTTG	TTTGGCTCTCTTTCTCCCTTG	FGSFSPL	FGSLSPL
1228	2528	AG	10	TACAGCATCGAACTTCCAAGC	TACAGCATCGGACTTCCAAGC	YSIELPS	YSIGLPS
1238	415	AG	9	TTTCTCGTGATTTTGCTTTTG	TTTCTCGTGGTTTTGCTTTTG	FLVILLL	FLVVLLL
1889	668	AG	10	ACACCCGGCCAGGAATTTACT	ACACCCGGCCGGGAATTTACT	TPGQEFT	TPGREFT
1889	685	AG	9	ACTTTTACAATTCGTAGGGGA	ACTTTTACAGTTCGTAGGGGA	TFTIRRG	TFTVRRG
1889	881	GA	10	CTAAATGTTAGAGGAAAAAGC	CTAAATGTTAAAGGAAAAAGC	LNVRGKS	LNVKGKS
1930	1379	AC	9	GAGGTTAGTAACCTTACAGCC	GAGGTTAGTCACCTTACAGCC	EVSNLTA	EVSHLTA
2023	574	GT	9	AGCTACACTGTGGCTTATGGA	AGCTACACTTTGGCTTATGGA	SYTVAYG	SYTLAYG
2023	602	CG	10	CCAGAACCTACTTGTCCTTGT	CCAGAACCTAGTTGTCCTTGT	PEPTCPC	PEPSCPC
2055	1540	CT	10	AAAAAATATGCTGAGGTTCTT	AAAAAATATGTTGAGGTTCTT	KKYAEVL	KKYVEVL
2055	1560	GC	9	AGACTGATAGGGAGACTTACG	AGACTGATACGGAGACTTACG	RLIGRLT	RLIRRLT
2055	1563	AG	9	CTGATAGGGAGACTTACGTTG	CTGATAGGGGGACTTACGTTG	LIGRLTL	LIGGLTL
2055	1617	GC	9	CAAGACTCCGAGCTAGACCAA	CAAGACTCCCAGCTAGACCAA	QDSELDQ	QDSQLDQ
2055	1680	GA	9	AGTCTGGTTGCTTTAGCACCA	AGTCTGGTTACTTTAGCACCA	SLVALAP	SLVTLAP
2055	1725	GA	9	ATCACGTTGGAAGTGTTGAAA	ATCACGTTGAAAGTGTTGAAA	ITLEVLK	ITLKVLK
2055	1987	TA	10	GTAACTGTGATGCAATGCCCC	GTAACTGTGAAGCAATGCCCC	VTVMQCP	VTVKQCP
2064	625	GC	9	TCAAATCAGGCTTCAGTTACT	TCAAATCAGCCTTCAGTTACT	SNQASVT	SNQPSVT

Table 3. List of Synonymous SNP coding data identified by Quality SNP

Contig no.	Position	SNP	Length	Normal sequence	Sequence with base change	Transcribed protein
361	358	GA	11	GCTAACCTGAGGCGCGCTGCT	GCTAACCTGAGACGCGCTGCT	ANLRRAA
361	454	CG	11	AGGCAGTTTCTCGGGCTGAGG	AGGCAGTTTCTGGGGCTGAGG	RQFLGLR
361	1053	AT	11	TACGGTTCGGCAGGCTATGCT	TACGGTTCGGCTGGCTATGCT	YGSAGYA
361	1189	TC	11	TCCATGGTGTCTACTGTTGCT	TCCATGGTGTCCACTGTTGCT	SMVSTVA
401	591	CA	11	GATGTTGTTGGCAGTCCATAC	GATGTTGTTGGAAGTCCATAC	DVVGSPY
401	609	AG	11	TACTATGTCGCACCAGAGGTG	TACTATGTCGCGCCAGAGGTG	YYVAPEV
401	618	GA	11	GCACCAGAGGTGTTGCGCAAG	GCACCAGAGGTATTGCGCAAG	APEVLRK
401	633	CT	11	CGCAAGCAGTACGGACCTGAA	CGCAAGCAGTATGGACCTGAA	RKQYGPE
401	678	TC	11	ATTTTGTATATTTTATTATCT	ATTTTGTATATCTTATTATCT	ILYILLS
401	699	AT	11	GGAGTGCCACCATTTTGGGCA	GGAGTGCCACCTTTTTGGGCA	GVPPFWA
401	837	GA	11	AATCCAACGAAGAGGCTATCA	AATCCAACGAAAAGGCTATCA	NPTKRLS
463	141	CT	11	GGAAAGTCGACCACTACTGGT	GGAAAGTCGACTACTACTGGT	GKSTTTG
468	1115	AT	11	ATTTCTACAGGAGCCTTCCTT	ATTTCTACAGGTGCCTTCCTT	ISTGAFL
567	427	CT	11	CCAAAGAAGACCGGCACCTCA	CCAAAGAAGACTGGCACCTCA	PKKTGTS
896	1490	AT	11	GAAAATGTGCTATATACGCAC	GAAAATGTGCTTTATACGCAC	ENVLYTH
899	1299	TC	11	CCCGAGCTTGTTAACAAGCTG	CCCGAGCTTGTCAACAAGCTG	PELVNKL
1136	285	CT	11	AAAATCAGAACCGTGGAGCTG	AAAATCAGAACTGTGGAGCTG	KIRTVEL
1228	2604	GA	11	AAGTCATTCACGTGTACTTTA	AAGTCATTCACATGTACTTTA	KSFTCTL

1233	636	TC	11	GTTTATAAGATTGAAGCTGAA	GTTTATAAGATCGAAGCTGAA	VYKIEAE
1889	608	CT	11	ATGCTTGACACCAAGGGTCCT	ATGCTTGACACTAAGGGTCCT	MLDTKGP
1889	813	AT	11	AAGTCCAAGACAGATGACTCT	AAGTCCAAGACTGATGACTCT	KSKTDDS
1889	912	TG	11	CCTTCCATCACTGAAAAGGAC	CCTTCCATCACGGAAAAGGAC	PSITEKD
2023	369	AG	11	GCTATGTTGTCACGCTCTGCG	GCTATGTTGTCGCGCTCTGCG	AMLSRSA
2023	378	GT	11	TCACGCTCTGCGGCAGGAATA	TCACGCTCTGCTGCAGGAATA	SRSAAGI
2055	1724	GA	11	CTAATCACGTTGGAAGTGTTG	CTAATCACGTTAGAAGTGTTG	LITLEVL
2055	1757	GA	11	TTACTTAGTCTGGTAACATCT	TTACTTAGTCTAGTAACATCT	LLSLVTS

Table 4. Comparative study of SNPs from Auto SNP and Quality SNP

Type of polymorphism	No. of polymorphisms in Auto SNP	No. of polymorphisms in Quality SNP
Transition	8827	67
Transversion	6840	54
Indels	2414	72
Total	18081	193

A similar computational analysis of SNP was carried out by Sakurai et al., (2013). Polymorphisms (SNPs and InDels) were discovered from the contig sequence alignment according to different criteria, Prochnik et al., 2012 fixed the criteria that at the contig should be able to align with the cassava draft genome sequence. Other criteria followed by researchers include that there were fewer than 3 other discontinuous nucleotide polymorphisms around 5 bp of a SNP site (Sakurai et al., 2013).

Validation of SNPs

Table 5. Genotypes used for validation of SNPs associated with CMD

Sl. No.	Resistant	Susceptible
1	Albert	CI732
2	96/1089A	CO2
3	Cr 11/43	Ambakadan
4	TME-3	Sree Vijaya
5	MNga-1	Sree Jaya

The 10 cassava genotypes/varieties used for validation of SNPs associated with CMD are presented in Table 5. Of the selected 5 SNP markers for primer synthesis, only forward primers were fluorescent labelled. Four different fluorescent dyes *viz*., 6-FAM, NED, VIC, PET were used. Validation of SNP was done by eluting the separated bands from the gel and then sequencing it. This sequence was aligned with the corresponding contig sequence from which the respective primer was designed. Multiple sequence alignment was done using ClustalX. The bands were eluted from the gel using the elution kit and were analyzed using 3500 capillary DNA Genetic Analyzer (Applied Biosystem). Three replicate analyses were carried out to avoid sequencing errors. These sequences were then aligned against their respective contigs using ClustalX. Sequence bands from the resistant genotype, MNga and susceptible genotype CI732 which contains the designed primers SNP896 and SNP1043 were sequenced. These sequences were aligned against contig 896 and contig1043 from which the primers were designed. ClustalX was used for multiple sequence alignment. The results showed that the sequence with SNP1043 did not show any variation in predicted SNP site, whereas SNP896 in MNga showed SNP at the 1493th position as designed but with a variation in the base. SNP896 did not show any change in that position for the susceptible variety CI732.

Conclusion

The study was aimed at developing molecular marker development, especially SNPs for cassava mosaicdisease resistance using bioinformatics tools and its validation. The preliminary data set for the identification of SSR/ SNP markers wasobtained from the EST section of NCBI (http://www.ncbi.nlm.nih.gov/nucest) andthe cassava transcript sequences (variety AM560-2, JGI annotation v4.1) from the Phytozome website (http://phytozome.jgi.doe.gov/pz/portal.html). With the help of these SNP prediction tools, we were able to develop novel markers which can be used for differentiating CMD resistant and susceptible genotypes. Primers were designed for both SNPs for CMD resistant genes, these primers were validated using 5 resistant and 5 susceptible cassava genotypes/varieties. Among the primers validated one SNP (SNP896) was able to clearly differentiate between the resistant and susceptible varieties. This is the first report of SNPs and SSRs computationally identified and verified in wet lab. In future, the identified 56 SNPs and 537 SSRs can be validated in wet lab and the resultingpotential markers can be utilized in the breeding program for screening CM Dresistance in cassava.

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Coat Protein Gene: A key tool for *Yam Mild Mosaic Virus* diagnosis in greater yam

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Abstract

Destruction of infected plants and use of virus-free planting materials are the common control measures used for preventing viral infections. These practices are inadequate to combat the virus attack in many incidents. Early detection of infection is an effective way to manage the systemic primary spread of viruses. Infection caused by *Yam mild mosaic virus* (YMMV), one of the major viruses in yams (*Dioscorea* spp) is difficult to detect in the early stages. Symptoms include mild mosaic, leaf mottling, and chlorosis and leaf distortion. In the present study, PCR was employed for the amplification of YMMV coat protein (CP) gene for diagnosing the virus from greater yam (*Dioscorea alata* L.) leaves. The specific primer pair was designed and validated for CP gene amplification, which yielded an amplicon of 810 bp in YMMV positive samples. Subsequent cloning in pUC18 vector and sequencing confirmed the presence of the full coat protein (CP). In addition to PCR-based diagnostic method, the accomplished isolation and characterization process opens avenues for generating virus-specific polyclonal antibodies through the utilization of the expressed coat protein. These antibodies can be further employed in serological techniques.

Keywords: YMMV, *Dioscorea alata,* PCR, Diagnosis, CP primer, cloning

Introduction

Yam is a common name for several species of *Dioscorea* which are tuberous starchy food cultivated and consumed in developing countries. They ranked as the third most important tuber crop after cassava and sweet potato (Fu et al., 2005). The most important edible yams are *D. alata*, *D. rotundata*, *D. esculenta* and *D.bulbifera*. Yams are essential for many tropical and subtropical livelihoods (Cao et al., 2021).The principal edible yams are cultivated mainly in three different regions such as Asia, Africa and South America and also the temperate regions (Lebot, 2009). They are vegetatively propagated crop and the species are characterized by weak climbing stem and underground tubers or rhizomes but some species produce aerial tubers too. These tubers are source of carbohydrate for millions of people around the world although some species are of medicinal and ornamental value (Padhan et al., 2002). In India greater yam is the important species, which is cultivated and consumed largely in the Southern and North Eastern states. Among them large number of wild yam species are utilized in the state of Kerala, where a dozen of them are consumed by the people belong to tribal community in the Wayanad district, which contains parts of Western Ghats (Balakrishnan et al., 2003). Yam can be stored longer than other fresh products which results in increased commercial value.

The yam production is adversely affected by pathogenic diseases (Amrutha et al., 2022). Virus diseases are of

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particular importance because the reduced vigor results in tuber yield loss and quality (Kolychikhina et al., 2021; Bakayoko et al., 2021). The prominent viruses that infect yams belong to the genera of *Potyvirus, Badnavirus, Cucumovirus, Potexvirus and Macluravirus* (Diouf et al., 2022). In India the reports about the presence of such viruses is scanty. A survey conducted by ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI) revealed the presence of *Yam mild mosaic virus* (YMMV) and *Yam mottling virus* (YMoV) (CTCRI, 2009). Diseases caused by viruses are hard to be controlled by the use of chemical applications unlike those caused by fungi and bacteria even though some techniques like hot water treatment found applicable in virus elimination from younger nodes (Jayaseelan et al., 2011).

Use of infected planting materials is a major means of spread of yam viruses and may restrict the international movement of selected germplasm due to quarantine restrictions. There is a requirement for testing planting materials for ensuring the exchange of virus free germplasm and for disease control. Management of yam virus disease is mainly through the principle of exclusion by using healthy planting materials and prevention of virus infection through cultivation of virus resistant varieties (Sastry et al*.,* 2014). Thus knowledge on accurate diagnosis necessitates sustainable yam production. In this study, we used PCR for the early detection of YMMV infection employing specifically designed primers.

Materials and Methods

Collection of sample

For identification of YMMV positive samples, leaves showing mild mosaic, leaf mottling, leaf distortion and chlorosis were collected from greater yamgrowing fields at ICAR-CTCRI (Fig.1). Asymptomatic samples showed no prominent symptoms except some mild indistinct chlorosis were also collected. This representative sample set was used for further test and analysis.

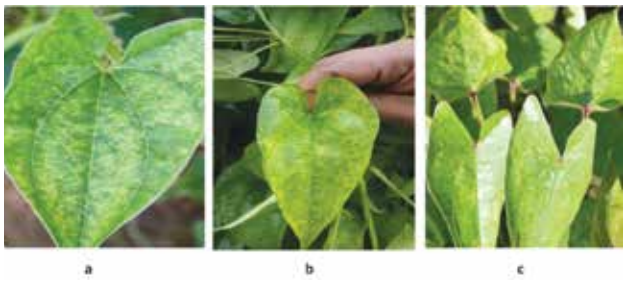

Fig. 1. Leaf samples showing mild mosaic (a), leaf mottling (b) and chlorosis (c)

RNA isolation and cDNA synthesis

Total RNA isolation was carried out using AmbionPurelink RNA Mini kit from the collected leaf samples of greater yam showing symptoms of various infection. The fully opened younger leaves were used. Younger yam leaves are most suitable than older leaves (Sika et al., 2015). The quantity and quality of the isolated RNA were measured on a NanoDrop spectrophotometer (DeNovix DS-11+ spectrophotometer) using RNase -free water as blank and performed an electrophoretic run on 1% agarose gel. Then visualized under UV to observe and document band for total RNA, typically show two major bands, corresponding to the 28S and 18S. This is because a reduced amount of tannins and phenolics in the younger leaves compared with the older leaves. Although the RNA yield obtained from leaf samples using AmbionPurelink RNA Mini kit method proved to be better method for good quality RNA.

Isolated RNA is less stable for long periods of storage; however, cDNA conversion ensures that the sample is not lost and was an essential prerequisite for conducting PCR based virus detection. From the isolated RNA, single-stranded cDNA was synthesized using Revert Aid FIRST strand cDNA synthesis kit (Thermo Scientific) with oligo dT primer. Approximately 2μg of total RNA was used in a 20 μL reaction with primers as described by the manufacturer's protocol. The reaction mix contain 4 μL of 5X Reaction Buffer, 1 μL of RiboLock RNase, Inhibitor (20 Uμ^{-1} L^{-1}), 2 μL of 10 mM dNTP Mix and 1 μL of RevertAid M-MuLV RT (200 Uμ^{-1} L^{-1}) and the volume was made up to 20 μL using Nuclease-free water. RNA was converted to cDNA using standard thermal conditions (single step reaction for 60 minutes at 42°C and the reaction were terminated by heating at 70°C for 5 min RNA was converted into cDNA for further PCR based screening. cDNA synthesis using Revert Aid FIRST strand cDNA synthesis kit was positive even from low quality RNA in most cases.

Amplification of partial coat protein gene

To identify YMMV positive samples, amplification of partial CP was performed using cDNA as a template and YMMV 1s 5'CACTCTTATGGTCTTGTT3'and YMMV 1c 5'TCTTATATGGTTCCTGTTC3' as forward and reverse primers (Sudheer, 2015), respectively. Amplification was done using (EmeraldAmp GT PCR Master Mix, Takara) in a thermocycler (BIO-RAD C1000 Touch) with initial denaturation of 4 min at 94°C followed by 35 cycles of denaturation at 94°C for 30 sec, annealing 52°C for 01 minutes and extension temperature 72°C for 1 min and final extension of 10 min at 72°C. To visualize a single band, an electrophoretic run was performed on a 1.5% agarose gel, followed by UV visualization for observation and documentation.

Designing and validation of full coat protein primers

Specific primer pair was designed by multiple alignments of sequences of different isolates of *Yam mild mosaic virus* available in NCBI database. They were designed for the amplification of conserved coat protein coding region with restriction sites EcoRI (GAATTC) and HindIII

(AAGCTT) for the ease of cloning. The primer sequences were checked for various parameters including annealing temperature, AT and GC content, primer dimer formation, and self-complementarity. The annealing temperature of the specific primer was optimized by using gradient PCR (BIO-RAD C1000 Touch) from 50-60°C, and the conditions for PCR amplification was standardized.

Amplification of full coat protein gene

YMMV positive samples confirmed through partial coat protein amplification were collected from the greater yam fields of ICAR-CTCRI and total RNA was extracted from fresh young, infected leaf tissue (100 mg). Amplification was done using EmeraldAmp GT PCR Master Mix (Takara) in a thermocycler (BIO-RAD C1000 Touch) with initial denaturation of 4 min at 94°C followed by 35 cycles of denaturation at 94°C for 40 sec, annealing 54°C for 70 seconds and extension temperature 72°C for 1 min and final extension of 10 min at 72°C. After confirming the presence of specific amplicon, PCR products were purified by gel elution kit (Nucleo spin gel and PCR clean-up Macherey- Nagel) and the quality was confirmed for cloning.

Cloning

The amplicon and the plasmid pUC18, restriction digested with EcoRI and HindIII (New England Biolabs) were purified and ligated into pUC 18 cloning vector. A 20 μl ligation reaction mix is designed to ligate insert and vector DNA molecules to form a recombinant molecule. The overnight incubation at 16°C provides optimal conditions for the ligation reaction. The mix contained insert DNA, vector DNA, T4 DNA ligase reaction buffer, and 1 μl of 5 unit $\mu^{-1}l^{-1}$ T4 DNA ligase (Thermo Scientific).

Transformation

200 μl of competent DH5 alpha cells was added to the ligation reaction mixture in the vial, which was kept in ice for 30 min prior to heat shock at a temperature of 42°C for 45 sec. in water bath. Immediately the vial was quenched into ice. 1 ml of Luria Bertani medium was added to the vial. After one hour of shaking incubation at 37°C, 100 μl cell suspension was spread on Luria Bertani Agar (LBA) medium containing Ampicillin (50 mg ml^{-1}) as selection marker, X-gal (20 mg ml^{-1}) and IPTG (40 μg ml^{-1}) for blue/white screening of recombinant colonies. Single white colonies were picked up from the Petri plate. Success rate of transformation is confirmed by running a colony PCR and a plasmid isolation followed by restriction digestion. Positive clones were selected and sequenced.

Results and Discussion

After first stranded cDNA synthesis, the detection of YMMV infection in all the samples were carried out using

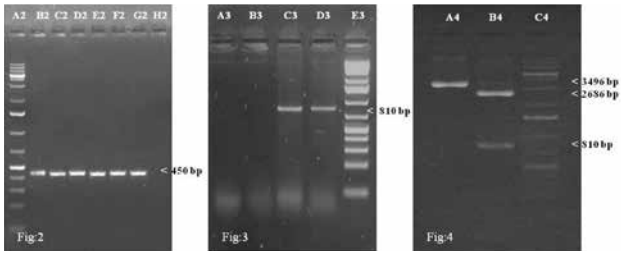

Fig. 2. (a) PCR amplification using YMMV lc and YMMV Is diagnostic primer yields a product size 450 bp (Lane B2-G2); (b) PCR amplification using forward Tcp(F) and reverse Tcp (R) primer to conform the full coat protein gene insert in the developed clone (Lane C3 & D3) showing 810 bp size PCR product marked against geneRuler 1kb plus DNA ladder (Lane E3) and (c) Gel image showing single digestion (LaneA4) of pUC 18 with insert, double digestion of pUC 18 (Lane B4) with 1 Kb pus ladder (Lane C4)

YMMV lc and YMMV ls specific primers, which amplifies the partial coat protein gene which yielded amplicons of 450 bp in samples positive for YMMV infection. There was no amplification observed in the non-template control which indicates that, no non- specific binding and primer dimer formation in PCR. The PCR results with YMMV lc and YMMV Is primers are shown in Fig. 2. The sequence results were initially analyzed and edited using BioEdit Sequence Alignment Editor Program version 7.2.5 and the obtained sequence was run through the online BLAST program of NCBI. The blast results query sequence of YMMV sequence showed maximum similarity 95% to *Yam mild mosaic virus* isolate DSMZ PV-1214 clone 1 polyprotein gene, (Accesssion no OM471977.1). A set of virus specific primers,Forward Tcp(F) primer 5'CCGAATTCGCAAGTAAGGAGCAG3' and Reverse Tcp(R) primer 5'GCAAGCTTGATATTACGCACTCC 3' which codes for the full length coat protein were designed and synthesized for the amplification of full CP gene of YMMV based on the most favorable combination of conserved regions in the multiple aligned nucleotide sequences. Primers were designed with restriction sites EcoRI and HindIII to ease the cloning procedure. The analysis of primers using program T_m Calculator (Thermo Scientific), revealed good GC content ideal annealing temperature, and also the designed primers did not exhibit hairpin formation and 3' complementarity. Performed a gradient PCR run with annealing temperature ranges from 50ºC to 60ºC to obtain the optimum annealing temperature and 54ºC for 70 sec. was the best for amplification. An amplicon of 810 bp was observed (Fig. 3) as a single band for virus positive samples previously diagnosed with primer YMMV lc and YMMV ls primer in 1.5 percentage agarose gel.

Viruses are of particular concern because, apart from causing significant reduction in tuber yield and quality, they restrict international exchange of germplasm. In this study, the samples were screened for their presence of

YMMV using nucleic acid based methods. PCR and RT-PCR techniques were employed to detect the presence of the virus.

In a preliminary study, screening to detect the virus was done using ELISA, it has major limitations such as its low sensitivity during periods of low titre. All leaf samples were also tested by PCR to ensure that plants with low virus load (Njukeng et al., 2002). In preliminary studies the samples screened through ELISA revealed that YMMV was the most common virus infecting greater yam. Similar observations of YMMV have been reported in *D. esculenta* from the Solomon Islands (Mumford and Seal, 1997). For confirmation of the presence of *Yam mild mosaic virus*, a published primer pair YMMV lc/YMMV 1s was used. PCR analysis with these primers yielded an expected product of size 450 bp. The newly designed TCP F/ TCPR in the study were used to amplify full CP gene (810 bp of) the virus from leaf samples of greater yam. The present investigation revealed the number of virus infections detected by PCR was more than that of the ELISA tests, possibly due to the high sensitivity of PCR. The lower sensitivity observed with the ELISA tests is similar to the findings of Mumford and Seal 1997 and could also be due to low virus concentration in yam (Brunt et al., 1990) or due to interference of polyphenols and glutinous polysaccharides contained in yam leaves (Rossel and Thottappilly, 1985). The development of primer targeting conserved regions in the genome is crucial for the detection of many viral variants (Davi et al., 2021). The transformed colonies harboring plasmids with the virus coat protein gene were analyzed for gene insert. Digestion of the plasmid with the restriction enzymes EcoRI and HindIII revealed a released insert of the expected size, 810 bp as seen in Fig. 4. The YMMV nucleotide sequence obtained in this studv showed maximum similarity of 98.03 % Yam mild mosaic virus isolate FIJI 3 polyprotein gene, (Accesssion AF548517.1) and 97.93% similarity to Yam mild mosaic virus Colombian isolate Col 2 polyprotein gene, partial cds (Accesssion AF548492.1).

Conclusion

Full coat protein gene of YMMV was amplified, sequenced, cloned and transformed for an Indian isolate which is related to the isolates from other countries. A PCR based diagnostic technique was standardized which is required for routine virus indexing and further the full CP amplicon could be utilized for developing viral coat protein leads to antibody production. This will prevent yield loss by the use of healthy planting material and safe exchange of germplasm.

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Co-pigmentation of sweet potato and greater yam anthocyanins with selected phenolic acids and its effect on *in vitro* antioxidant activity

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Abstract

The effect of co-pigmentation on the antioxidant capacity of anthocyanins isolated from purple colored root tubers of greater yam (*Dioscorea alata*, Acc. Da-340) and sweet potato (*Ipomoea batatas*, *cv* Bhu Krishna) with caffeic, ferulic and p-coumaric acids was investigated in this study. The DPPH radical scavenging activity of both the anthocyanins enhanced significantly after co-pigmentation. However, phenolic acids behaved differently with different concentrations of anthocyanins. Except at very low anthocyanin concentrations, caffeic acid and ferulic acid served as effective co-pigments for greater yam and sweet potato anthocyanins respectively, leading to an increase in antioxidant potential. At very low concentrations of the pigment and co-pigment used, the effect was reverse. The highest % radical scavenging activity was observed for greater yam anthocyanins at a concentration of 6 μgml^{-1} and 26 μg ml^{-1} of ferulic acid as the co-pigment, followed by the same concentration of anthocyanins and 24 μg ml^{-1} of caffeic acid. P-coumaric acid was not as effective as caffeic and ferulic acids. This study indicated the existence of some distinct intermolecular interactions that ensue in the complex framework of natural colors and the results could beuseful in designing bioactive food colorants.

Keywords: Greater yam, Sweet potato, Anthocyanins, Phenolic acids, Co-pigmentation, Radical scavenging activity

Introduction

Anthocyanins are naturally occurring plant phenolics consisting of an aglycone linked to one or more sugar moieties that can be further acylated by aromatic or aliphatic organic acids. Anthocyanins are potential anticancer agents and have the capacity to scavenge active radicals to prevent carcinogenesis (Lila, 2004; Wang & Stoner, 2008). These are also capable of improving visual functions (Khoo, Azlan, Tang, & Lim, 2017; Shim, Kim, Choi, Kim, & Park, 2012) and inhibiting platelet aggregation (Song et al., 2014; Yang et al., 2010). Anthocyanins of purple sweet potato are noted for their stability and physiological functions. Anthocyanins of a purple sweet potato cultivar named Bhu Krishna contain peonidin and cyanidin derivatives, which have anti-proliferate effects on breast, colon and cervical cancer cells (Vishnu et al., 2019). Greater yam (*Dioscorea alata*) tuber anthocyanins were found to be rich in cyanidin derivatives and exhibit high antioxidant activity (Moriya et al., 2015).

Co-pigmentation is the formation of non-covalent complexes due to the interaction between anthocyanins/ anthocyanin derived pigment with a co-pigment, which also changes the optical properties of the pigment (Trouillas et al., 2016). A co-pigment is a compound which can enhance the color of anthocyanin solution

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due to the intermolecular interactions between them (Welch, Wu, & Simon, 2008). Generally, co-pigments have extended π-conjugated systems that favour π-π stacking interaction and have hydrogen bond donors or acceptors like hydroxyl or carbonyl groups. Phenolic acids can serve as co-pigments due to the presence of aromatic rings and hydroxyl groups. Intermolecular co-pigmentation is possible between anthocyanin and phenolic acids, polyphenolic compounds, metal ions, etc. The vertical hydrophobic stacking of the aromatic nuclei is possible between anthocyanins and polyphenolic compounds. The co-pigmentation of anthocyanins with other compounds is responsible for the final colour of anthocyanins.

The factors that affect the co-pigmentation are the nature of aglycone and co-pigment, anthocyanin concentration, the molar ratio between anthocyanin and co-pigment, pH, type of solvent used and temperature (Trouillas et al., 2016). Several models have been proposed for the intermolecular co-pigmentation between differently acylated anthocyanins and other aromatic molecules (Gauche, Malagoli, Terezinha, & Luiz, 2010; Kopjar and Piližota, 2009; Trouillas et al., 2016). Anthocyanins themselves can act as co-pigment due to self-association, but it is less efficient than co-pigmentation with phenolic acids and their derivatives. The force acting behind self-association was the hydrophobic interaction between the aromatic nuclei, which are stacked parallel to each other. The previous reports said that vertical stacking by π-π interactions is more prominent than horizontal stacking by hydrogen bonds (Trouillas et al., 2016). However, there is little information available on how co-pigmentation with phenolic acids leads to changes in the antioxidant activity of anthocyanins. Therefore, this study was undertaken to understand the effect of intermolecular co-pigmentation of anthocyanins isolated from the purple flesh tubers of sweet potato and greater yam with some selected phenolic acids on their antioxidant capacity. The root tubers of anthocyanin rich promising sweet potato cultivar Bhu Krishna and a greater yam accession, Da-340, which are available in the collection of ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI), Thiruvananthapuram, Kerala, India were used as anthocyanins sources.

Materials and Methods

Materials

Freshly harvested tubers of greater yam (Acc. Da-340) and sweet potato (*cv* Bhu Krishna) were collected from the ICAR-CTCRI experimental farm. The tubers were washed thoroughly, sliced and subjected to solvent extraction for isolating the anthocyanins. The phenolic acids used were hydroxy cinnamic acids *viz*., caffeic, ferulic and p-coumaric acids.Amberlite XAD-7, 1,1-Diphenyl-2-picryl-hydrazil (DPPH),ethyl acetate, trifluoroacetic acidand methanol were purchased from Merck India Ltd. Citric acid, sodium citrate, ethanol, hydrochloric acid, sodium hydroxide and potassium hydroxide were of highest analytical grade. Caffeic acid, ferulic acid and p-coumaric acid were purchased from Sigma Aldrich (St. Louis, USA).

Isolation and purification of anthocyanins

Anthocyanins were extracted from a weighed quantity (100g) of the fresh tubers using methanol, acidified with 0.5% triflouroacetic acid (TFA). The extraction was continued until the residue was colourless. The filtrates were combined and concentrated in a rotary flash evaporator (Buchi Multivapor P-6) at 30°C under reduced pressure. The crude anthocyanin pigment was then dissolved in distilled water and partitioned with ethyl acetate to remove other non-polar compounds. The remaining aqueous layer was collected and subjected to column chromatography using Amberlite XAD-7 resin. The anthocyanins adsorbed on Amberlite were eluted using methanol acidified with 0.5% TFA. The eluent was collected, pooled and concentrated using a rotary flash evaporator at 30°C under reduced pressure and then lyophilized to obtain purified anthocyanins.

Co-pigmentation of anthocyanins

The anthocyanins were prepared at a concentration of 2.23×10^{-3} mmol ml^{-1} of cyanidin-3-O-glycoside equivalent. The concentration of the co-pigments, *viz.,* caffeic, ferulic and p-coumaric acids were made to millimolar equivalent or proportional to the fixed concentration of anthocyanins.The citrate buffer at pH 3.5 was used as a solvent for all the co-pigmentation studies. The preliminary screening for determining the extent of co-pigmentation was done at different molar ratio of anthocyanins with selected phenolic acids (1:1, 1:5, 1:10, 1:20, 1:30 and 1:40). The shift in λ_{max} and increase in absorbnce was analyzed in each case and the ratio at which large increase in absorbance was considered as the effective co-pigmentation ratio and these concentrations were selected for further studies.

UV-Visible spectroscopy

Spectroscopic evaluation of purified anthocyanins and co-pigmented anthocyanins was done by using a UV-Vis spectrophotometer (Perkin Elmer, Lambda 25, Switzerland). The samples were scanned in a wavelength region of 400-700 nm and the absorbance was also recorded at the maximum wavelength (λ_{max}). The difference in absorbance at λ_{max} of anthocyanins before and after co-pigmentation was expressed as ΔA.

Evaluation of antioxidant activity by DPPH assay

A known weight of anthocyanins was dissolved in citrate buffer solution (pH 3.5) and used for preparing the test solution. Free radical scavenging activity of the anthocyanins of greater yam and sweet potato was

measured by 2, 2- diphenyl-1-picryl hydrazyl (DPPH) assay. Briefly, 0.2 mM solution of DPPH in methanol (1 ml) was added to anthocyanin solution of different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then, absorbance was measured at 517nm using a spectrophotometer (UV-Vis Perkin Elmer, Lambda 25, Switzerland). The IC_{50} value of anthocyanins, which is the concentration of sample required to inhibit 50% of the DPPH free radicals, was calculated using the Log dose inhibition curve. The percent DPPH scavenging effect was calculated by using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A0\text{-}A1}{A0} \times 100$$

Where, A_0 was the absorbance of the control, which contains DPPH alone, and A1 was the absorbance of anthocyanins.

Evaluation of type of interaction

The type of interaction between the anthocyanins and the co-pigments was evaluated by comparing the theoretical and experimental (real) radical scavenging activities. The theoretical radical scavenging activity (RSA) was calculated by adding the individual radical scavenging activities of pigment and co-pigment at a particular concentration. Real RSA was experimentally determined as per the protocol mentioned in the previous section by using the same combination of pigment and co-pigment which were taken for calculating theoretical radical scavenging activity. If real RSA was greater than that of theoretical RSA, a positive effect occurred and otherwise the effect was considered as negative.

Statistical analysis

Single-factor analysis of variance (ANOVA) of data was performed using the package SAS 9.3. Duncan Multiple Range Test (DMRT) was done for the pair-wise comparison of the mean values.

Results and Discussion

Effect of co-pigmentation on UV-Vis spectra of anthocyanins

Co-pigmentation can be identified by analyzing the change in colour intensity and spectral shifts observed by UV-visible spectroscopy, which is used widely for identifying the co-pigmentation because only low pigment concentration is needed for it (Trouillas et al., 2016). It is possible to evaluate co-pigmentation interms of bathochromic or hyperchromic shifts. The UV-vis spectra of co-pigmented greater yam and sweet potato anthocyanins are shown in Fig. 2A and Fig. 2B respectively. The greater yam and sweet potato anthocyanins showed wavelength of the maximum absorbance (λ_{max}) at 528.97 nm and 527.43 nm, respectively at a concentration of 2.23×10^{-3} mmolml^{-1} (Table 1). These peaks in the visible region are considered the characteristic peaks of anthocyanins and are responsible for their deep red colour. Anthocyanin color may vary according to the number and position of hydroxyl groups. The distinctive peak of anthocyanins generally ranges from 450-560 nm and is due to the B-ring of anthocyanins (Figure 1). Another characteristic band in the UV-visible spectrum at 240-280 nm is due to the A-ring, called the benzoyl system. The evidence of positive co-pigmentation can be identified by evaluating the increase in absorbance (ΔA) and the shift in wavelength (λ_{max}). A significant change in absorbance (hyperchromic shift) was observed after co-pigmentation of greater yam and sweet potato anthocyanins with caffeic acid and ferulic acid at a pigment/co-pigment ratio of 1:10. However, in the case of p-coumaric acid, the hyperchromic shift was observed at the pigment/co-pigment ratio of 1:20 (Table 1). Co-pigmentation interaction of anthocyanins with phenolic acids resulted in the presence of hydroxyl group in the aromatic ring.

Co-pigmentation of purple yam anthocyanins with caffeic acid resulted in an increase in absorbance of the former by 56.10 % ($\Delta A = 0.087$) (Table 1), which was observed as the most effective co-pigmentation in greater yam tuber anthocyanins in the present study. However, p-coumaric acid was found to be a less effective co-pigment with greater yam anthocyanins by virtue of the minimal increase in absorbance ($\Delta A = 0.046$) after co-pigmentation with it. Ferulic acid showed about a 45.80% increase in absorbance with an ΔA value of 0.071. The two important factors that are expected behind these observations are π-π stacking and intermolecular hydrogen bonding. Cyanidin and peonidin are reported to be the common aglycones present in greater yam anthocyanins and among these, cyanidin was the major one (Moriya et al., 2015; Shoyama et al., 1990). Cyanidin contains two hydroxyl groups in the B ring (Figure 1) and can effectively participate in intermolecular hydrogen bonding and π-π stacking with the co-pigment. Caffeic acid, which also contains two hydroxyl groups was able to participate in co-pigmentation interaction with cyanidin-rich greater yam anthocyanins with identical symmetry, more effectively than ferulic and p-coumaric acids, both of which contain only one hydroxyl group attached to the aromatic ring.

Co-pigmentation of sweet potato anthocyanins with ferulic acid resulted in an increase in absorption intensity with a A value of 0.103, which was observed as the most effective co-pigmentation among the three selected phenolic acids (Table 1). This increase in absorbance was 84.4% greater than that of individual sweet potato anthocyanins. Here, unlike the case with greater yam anthocyanins, caffeic acid was the least effective co-pigment ($\Delta A = 0.046$). Co-pigmentation of anthocyanins with p-coumaric acid showed an increase in absorbance value by 57.37% at a molar ratio of 1:20.

Table 1. UV-visible absorbance* and λ_{max} of anthocyanins before and after co-pigmentation with phenolic acids

Anthocyanin source	Co-pigment	Molar ratio of anthocyanins/ Co-pigment	λ_{max} (nm)	Absorbance at λ_{max}	ΔA	$\Delta\lambda_{max}$ (nm)
Greater yam tuber	-	-	528.97	0.155 ± 0.03^{f}	-	-
	Ferulic acid	1:10	529.74	0.226 ± 0.05^{b}	0.071	0.77
	Caffeic acid	1:10	529.44	0.242 ± 0.03^{a}	0.087	0.47
	p-Coumaric acid	1:20	529.10	0.201 ± 0.06^{c}	0.046	0.13
Sweet potato tuber	-	-	527.43	0.122 ± 0.04^{g}	-	-
	Ferulic acid	1:10	528.54	0.225 ± 0.07^{b}	0.103	1.11
	Caffeic acid	1:10	528.24	0.168 ± 0.05^{e}	0.046	0.81
	p-Coumaric acid	1:20	528.46	0.192 ± 0.05^{d}	0.070	1.03

*Mean values with similar alphabets in the superscript are not significantly different

Sweet potato tubers contain acylated anthocyanins with peonidin and cyanidin aglycones, and among these peonidin was reported as the major aglycone (Cuevas et al., 2011; Montilla et al., 2010; Sun et al., 2014; Vishnu et al., 2019). A previous study revealed that the major compound present in the tuber anthocyanins of sweet potato cultivar Bhu Krishna are caffeoyl derivatives of peonidin (Vishnu et al., 2019). The substituents attached to the B ring of aglycone is responsible for the extent of stacking with co-pigments, which leads to the intermolecular co-pigmentation and increase in color intensity of anthocyanins (Boulton, 2001b; Eiro and Heinonen, 2002; Kammerer, 2016; Trouillas et al., 2016; Welch et al., 2008). The comparatively lower co-pigmentation efficiency of caffeic acid towards sweet potato anthocyanins could be explained as follows. Instead of stacking with the B-ring of the aglycone, the stacking of caffeic acid takes place with the structurally similar caffeoyil group attached to the sugar moiety present in the sweet potato anthocyanins. However, the aforementioned stacking was irrelevant tothe increase in color intensity and stability of anthocyanins.Peonidin contains one each of the -OH and -OCH_3 groups in the B-ring. Similar groups are present in ferulic acid and hence it can serve as an effective co-pigment through π-π stacking with sweet potato anthocyanins. This result is also in agreement with those of the previous studies, which reported that hydroxycinnamic acids and its derivatives are more effective co-pigments than benzoic acid and its derivatives (Marković, et al., 2000; Malaj et al., 2013; Trouillas et al., 2016). P-coumaric acid was observed as a more effective co-pigment for sweet potato tuber anthocyanins than that for greater yam anthocyanins.

The co-pigmentation effects of greater yam and sweet potato anthocyanins with the three selected phenolic acids were found distinctive. Based on change in absorbance, caffeic acid was found to be the best co-pigment for cyanidin-rich greater yam anthocyanins, but the same was identified as the least potent co-pigment with sweet potato anthocyanins with peonidin as the major aglycone. The results of the study revealed that the structure of aglycone and the acyl group attached to anthocyanins plays an important role in the co-pigmentation reaction.

DPPH assay of anthocyanins

The antioxidant activity of a sample is due to its hydrogen-donating ability. When chemical substances or biological structures interact with each other, it results in an overall effect that is greater than the sum of the individual effects of any of them and is termed as synergistic effect (Boulton, 2001a; Palafox-Carlos et al., 2012). When co-pigmentation results in a radical scavenging activity (real or experimental), which is greater than theoretical activity, it is termed as positive co-pigmentation.

The individual antioxidant activity of anthocyanins in the tubers of greater yam and sweet potato was determined and the results are presented as percentage radical scavenging capacity (% RSA) in Table 2. Greater yam anthocyanins were found to have the greater antioxidant capacity with an IC_{50} value of 8.72 $\mu g ml^{-1}$, than sweet potato anthocyanins, which have an IC_{50} value of 11.58 $\mu g ml^{-1}$ (Table 2). This could be attributed to the presence of higher levels of potentially bioactive cyanidin content in greater yam anthocyanins when compared to that in sweet potato anthocyanins (Cuevas et al., 2011; Moriya et al., 2015; Shoyama et al., 1990; Vishnu et al., 2019).

Table 2. DPPH radical scavenging activity[1] of sweet potato and greater yam tuber anthocyanins and different phenolic acids[2]

	Concentration ($\mu g\ ml^{-1}$)	% RSA[3]	IC_{50} ($\mu g\ ml^{-1}$)
Anthocyanin source			
Greater yam tuber anthocyanins	1.5	16.5 ± 0.35^{e}	8.92^{b}
	3	25.8 ± 0.56^{d}	
	6	36.8 ± 0.75^{b}	
	12	58.1 ± 0.97^{a}	

Sweet potato tuber anthocyanins	1.5	7.9 ± 0.54[g]	11.58[a]
	3	12.6 ± 0.86[f]	
	6	16.8 ± 1.23[e]	
	12	33.8 ± 1.66[c]	
Phenolic acid			
Caffeic acid	6	6.2±0.39[i]	63.54[c]
	12	11.6±0.78[g]	
	24	19.6±0.72[e]	
	48	38.5±1.13[a]	
Ferulic acid	6.5	8.3 ± 0.55[h]	70.28[b]
	13	14.8 ±0.61[f]	
	26	22.2 ± 0.95[c]	
	52	37.4 ± 1.37[b]	
P-Coumaric acid	11	3.2±0.54[j]	108.75[a]
	22	8.6± 0.66[h]	
	44	20.8± 1.13[d]	
	88	38.9± 1.56[a]	

[1] Mean values with similar alphabets in the superscript are not significant different.

[2] Concentration of phenolic acids used was proportional to 2.22×10^{-3} mmol of cyanidin-3-O-glycoside.

[3] RSA - Radical scavenging activity.

Individual antioxidant potential of caffeic, ferulic and p-coumaric acids were also estimated to understand their biological action. Among these, caffeic acid had comparatively higher DPPH radical scavenging activity (IC_{50}- 63.54 μg ml^{-1}) than the other two (Table 2). The activity was lowest for p-coumaric acid (IC_{50} - 108.75 μg ml^{-1}). The antioxidant activity of greater yam and sweet potato anthocyanins, after co-pigmentation with phenolic acids, is presented in Tables 3 and 4, respectively and the activity was found to be dose-dependent, *i.e.*, the activity increased with increase in concentration.

Co-pigmentation of greater yam anthocyanins with caffeic, ferulic and p-coumaric acids resulted in an increase in experimental values of % RSA in comparison to the theoretical values, except at the highest anthocyanin concentration of 12 μg ml^{-1} (Table 3). At the highest anthocyanin concentration of 12 μg ml^{-1}, a negative effect was observed with a decrease in the experimental % RSA value when compared to the theoretical value. The results also revealed that at higher concentrations of greater yam anthocyanins (3-12 μg ml^{-1}), co-pigmentation with ferulic acid resulted in comparatively higher antioxidant activity than that with caffeic acid and p-coumaric acid. The highest real % RSA was observed for greater yam anthocyanins at a concentration of 6 μg ml^{-1} and 26 μg ml^{-1} of ferulic acid as the co-pigment, followed by the same concentration of anthocyanins and 24 μg ml^{-1} of caffeic acid.

The sweet potato anthocyanins showed positive interaction with caffeic acid and p-coumaric acid at all concentrations with an increase in real % RSA when compared to the theoretical values (Table 4). However, with ferulic acid, the interaction was negative at the highest concentration of anthocyanins (12 μg ml^{-1}). An interesting factor noted was that at the highest concentration of anthocyanins, caffeic acid showed significantly higher antioxidant activity than other phenolic acids. Even though ferulic acid served as an excellent co-pigment for sweet potato anthocyanins, with caffeic acid, especially at higher concentrations, these anthocyanins exhibited

Table 3. The DPPH radical scavenging activity* of greater yam anthocyanins co-pigmented with phenolic acids

Sample	Concentration (μg ml^{-1})		% RSA		Type of interaction
	Anthocyanins	Caffeic acid	Real	Theoretical	
Greater yam anthocyanins + caffeic acid (1:10 mM ratio)	1.5	6	32.3±0.91[j]	22.7±0.74[g]	Positive
	3	12	48.6±1.21[h]	37.4±1.34[d]	Positive
	6	24	71.8±0.83[e]	56.4±1.47[b]	Positive
	12	48	88.4±2.34[c]	96.6±2.10[a]	Negative
Greater yam anthocyanins + ferulic acid (1:10 mM ratio)	1.5	6.5	27.6± 0.98[k]	24.8 ± 0.90[f]	Positive
	3	13	50.4± 1.11[g]	40.6 ± 1.17[c]	Positive
	6	26	77.06±1.33[d]	59.0 ± 1.70[b]	Positive
	12	52	91.98±0.93[a]	95.5 ± 2.34[a]	Negative
Greater yam anthocyanins + p-coumaric acid (1:20 mM ratio)	1.5	11	22.7±0.48[l]	19.7±0.89[h]	Positive
	3	22	35.4±0.81[i]	34.4±1.22[e]	Positive
	6	44	61.8±0.93[f]	57.6±1.88[b]	Positive
	12	88	90.1±1.34[b]	97.0±2.53[a]	Negative

*Mean values with similar alphabets in the superscript are not significantly different.

Table 4. The DPPH radical scavenging activity* of sweet potato tuber anthocyanins co-pigmented with phenolic acids

Sample	Concentration (μg ml^{-1})		% RSA		Type of interaction
	Anthocyanins	Caffeic acid	Real	Theoretical	
Sweet potato anthocyanins + caffeic acid (1:10 mM ratio)	1.5	6	16.2±0.43^{i}	14.1±0.93^{g}	Positive
	3	12	31.2±0.83^{g}	24.2±1.64^{e}	Positive
	6	24	46.8±1.43^{d}	36.4±1.95^{c}	Positive
	12	48	94.4±2.43^{a}	72.3±2.79^{a}	Positive
Sweet potato anthocyanins + ferulic acid (1:10 mM ratio)	1.5	6.5	22.9±0.78^{h}	16.2±1.09^{g}	Positive
	3	13	31.8±1.11^{g}	27.4±1.47^{d}	Positive
	6	26	44.0±1.39^{e}	39.0±2.18^{b}	Positive
	12	52	70.6±1.53^{c}	71.2±3.03^{a}	Negative
Sweet potato anthocyanins + p-coumaric acid (1:20 mM ratio)	1.5	11	14.2±0.18^{j}	11.1±1.08^{h}	Positive
	3	22	23.1±0.81^{h}	21.2±1.52^{f}	Positive
	6	44	39.0±0.79^{f}	37.6±2.36^{bc}	Positive
	12	88	74.9±0.53^{b}	72.7±3.23^{a}	Positive

*Mean values with similar alphabets in the superscript are not significantly different.

greater antioxidant activity. When comparing the effect of co-pigmentation of caffeic acid with greater yam and sweet potato anthocyanins, at lower concentrations of 1.5, 3 and 6 μg ml^{-1}, the co-pigmentation increased the %RSA of greater yam anthocyanins more than that of sweet potato anthocyanins. Although at the highest concentration of caffeic acid, a positive interaction that leads to an increase in antioxidant activity was observed for sweet potato anthocyanins. However, under the same conditions, a negative interaction that leads to a decrease in real %RSA was observed for greater yam anthocyanins. The effect of co-pigmentation with ferulic acid on the antioxidant activity was higher in sweet potato anthocyanins than that of greater yam anthocyanins at a concentration of 1.5 μg ml^{-1}. However, at all the other selected concentrations of anthocyanins, the activity was more with sweet potato anthocyanins. When comparing all three phenolic acids, p-coumaric acid was the least potent to increase the antioxidant potential of both anthocyanins.

From the above results, it is clear that caffeic acid can serve as an effective co-pigment with cyanidin rich greater yam anthocyanins only at low anthocyanin concentrations. This was attributed to the formation of more stable aglycone after co-pigmentation with caffeic acid due to the increased intermolecular hydrogen bonding and π-π stacking, which enhanced the antioxidant potential. However, at low concentrations of sweet potato anthocyanins, ferulic acid was found to be a more effective co-pigment. The stacking between sweet potato anthocyanins and ferulic acid enhances the stability of B-ring of peonidin-rich anthocyanins which also stabilizes the flavylium cation resulting in an increase in antioxidant activity.

Previous reports revealed that anthocyanins in greater yam were found as mostly acylated with sinapic and ferulic acids (Moriya et al., 2015). Sinapic acid contains two methoxy and one hydroxyl group, while ferulic acid contains one methoxy and one hydroxyl group. At low anthocyanin concentrations, the co-pigment, ferulic acid can stack with the structurally similar acyl moiety present in greater yam anthocyanins, while sufficient co-pigment molecules might not be available to interact with the B-ring of the aglycone. When the concentration of pigment and co-pigment increases, more co-pigment molecules were expected to stack with the B-ring of aglycone. On the other hand, sweet potato anthocyanins are reported to be acylated with caffeic acid (Vishnu et al., 2019). At low anthocyanin concentrations, the caffeic acid co-pigment added can stack with the structurally similar caffeoyl moiety present in the sweet potato anthocyanins. Therefore, here the interaction of co-pigment with the B-ring of aglycone might be minimal. Caffeic acid can stack with the B-ring of sweet potato anthocyanins effectively at still higher anthocyanin and co-pigment concentrations.

Positive interaction which leads to an increase in antioxidant potential may be mainly due to the increase in the availability of the coloured flavylium cation which was converted back from colourless hemi-ketal form during the addition of the co-pigment. However, at higher concentrations, the decrease in experimental radical scavenging activity might be due to the increase in intramolecular co-pigmentation. According to previous

studies, intramolecular co-pigmentation of anthocyanins was less effective than intermolecular co-pigmentation (Trouillas et al., 2016). The structure of anthocyanins present in both the tuber was highly influencing the change in antioxidant potential after co-pigmentation. The co-pigmentation of anthocyanins with phenolic acids is also responsible for the conversion of less active hemiketal structure to bioactive flavylium cation.

Recently, co-pigmentation has a remarkable interest in food industry because of the opportunities to design unique combinations of natural pigment and co-pigment with finely harmonized stable colors coupled with enhanced bioactivity. Anthocyanins and anthocyanidins can be used as a natural food colorants not only due to the high color intensity but also due to their prominent nutraceutical properties which is responsible for potential health benefits (Khoo et al., 2017). Most of the food products such as fruits, vegetables, wine, cocoa and tea contain high concentrations of hydroxy cinnamic acids having potent anti-oxidant, anti-inflammatory, anti-diabetic and anti-hyperlipidemic properties (Alam et al., 2016; El-Seedi et al., 2018). These two classes of compounds, *viz*., the non-toxic, bioactive anthocyanins and phenolic acids (hydroxycinnamic acids) together can contribute to an enhanced color intensity and antioxidant potential than that of their individual contributions. According to the results of the present study, the color intensity and antioxidant potential of greater yam and sweet potato tuber anthocyanins could be enhanced by co-pigmentation with hydroxycinnamic acids such as caffeic and ferulic acids.

Conclusions

Greater yam and sweet potato tuber anthocyanins exhibited a positive co-pigmentation with phenolic acids resulting in an increase in antioxidant activity at lower concentrations. Ferulic acid and caffeic acid served as effective co-pigments and lead to a significant increase in antioxidant potential of both the anthocyanins. The co-pigmentation efficiency of p-coumaric acid was less when compared to the other two phenolic acids. Very high concentration of anthocyanins caused a negative interaction in most cases. This study proved that the structure of aglycone, presence of acylation and concentration of anthocyanins as well as phenolic acids can affect the antioxidant potential of anthocyanins. In the light of these results, the importance of choosing the best combination of anthocyanins and phenolic acid is important in increasing the colour intensity and antioxidant potential of anthocyanins in functional foods.

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Tillage and mulching practices in cassava (*Manihot esculenta* Crantz): Influence on soil carbon mineralization, enzyme activity and glomalin content

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Abstract

A study was undertaken in laterite soils (Ultisols) in 2017 to find out the effects of continuous adoption of different tillage and mulching practices on carbon and nitrogen mineralization. Soil physico-chemical properties and biological parameters *viz*., soil enzyme (as an indicator of soil biological activity) and glomalin content (as an indicator of soil carbon sequestration) were estimated to find the relationships with the mineralization rate. Surface soil samples (0-0.15 m) from five treatments involving four tillage and mulching treatments each with three replications and a control were taken for the study. Results showed that maximum water holding capacity (WHC) of 41.3% was found in soils under conventional tillage with sheet mulching (T1) as compared to 37.7% in control (T5) with optimum BD (1.41 Mg m^{-3}) and porosity (46%). No significant differences in soil pH were observed among tillage practices. The soil organic carbon registered a maximum value under T5 (1.37%). A significant increase of carbon mineralization was noted in T1 (137.7 mg CO_2 100 g^{-1} soil) followed by minimum tillage with mulching (T3). The maximum mineralization of nitrogen, dehydrogenase activity, total glomalin (TG) and easily extractable glomalin (EEG) were recorded with control (T5). Results indicated that adoption of conventional tillage with porous ground cover sheet mulching practices increased soil carbon mineralization activity to an extent of 18.2, 19.3 and 28.6% over minimum tillage with mulching at 24 h, 48 h and 7 days, respectively. Among the soil properties, conventional tillage practices increased the soil bulk density insignificantly and the water holding capacity to an extent of 15.6% over minimum tillage.

Keywords: Minimum tillage, Soil Glomalin, Carbon mineralization, Soil dehydrogenases

Introduction

Conservation tillage system has a primary objective on reduction of soil and water losses due to reduced traffic operations, thereby decreasing soil compaction and costs for labour and other equipment. In soil ecosystems, the rate of organic matter decomposition, soil microbial enzymatic activity and carbon sequestration are interrelated to each other, and the rates are determined by initial soil management practices such as types of tillage such as conventional tillage, minimum tillage and mulch tillage etc., as well as fertilizer rates and applications of other amendments.

Organic matter decomposition, especially the process of carbon mineralization in soils is the most important ecological process mediating energy flux and nutrient cycling in terrestrial ecosystems. It has been attributed

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that this process is critically linked to the diversity of enzymes produced by the microbial community. Specially, dehydrogenase activity in soil provides correlative information on the soil metabolic activity corresponds to microbial populations. Soil microbes play a key role in the microbial process of nutrient cycling that involves soil organic component or compounds fragmentation, distribution and mineralization. This is closely linked with the build-up and maintenance of organic matter content and physical structure of soils, soil plant relations and decides the productivity capacity of soils. It is argued and even proved that soil microbiological activity indicators, soil glomalin content are quite sensitive and they can be successfully used in soil sustainability studies.

Glomalin-related soil protein (GRSP) can be produced specifically by Arbuscular Mycorrhizal Fungus (AMF). The efficient glomalin-producing AMFs include *Acaulosporamorroaiae, Glomus luteum, Glomus verruculosum, and Glomus versiforme*. A glycoprotein called glomalin, which contains 30 and 40 percent carbon (C), has been found to be enduring and stable in soil. Due to its high carbon content and aggregate stability, glomalin can sequester more carbon in the soil. In terrestrial ecosystems, higher aggregate stability promotes high organic carbon conservation (Hossain, 2021). To increase soil aggregation, glomalin, and soil organic carbon (SOC) content, zero tillage is recommended combined with crop residue retention and diverse cropping. Glomalin serves an important role in soil aggregation as stable glue (Wright et al., 2007).

Agricultural productivity depends both on management practices as well as soil microbiological dynamics, which in turn is affected by the former. In India, cassava is a major starchy as well as edible root crop and is cultivated in an area of 1.83 lakh ha and with a tuber production of 6.941 lakh tones in the year 2021 (based on estimates of FAO) in major soil type's *viz.,* Alfisols, Ultisols and Vertisols predominantly in the states of Kerala and Tamil Nadu. Farmers follow varied adoption of conventional tillage practices with 3-4 initial ploughings, which leads to abnormal mineralization of C and N and resulting in gaseous loss as CO_2 into the atmosphere. Besides, there are well established indicators of soil carbon sequestration as measured by the content of soil Glomalin (Total Glomalin (TG)) and easily extractable Glomalin (EEG), which are not exploited and adequately researched in India in general and laterite soils subjected to continuous cultivation of these practices, particularly in cassava. Therefore, this study was conducted to assess the effects of tillage on soil mineralization of carbon and nitrogen as well as their interaction with soil properties especially glomalin content.

Materials and Methods

The field work was undertaken at the farm of ICAR-CTCRI as part of study in the third year under the institute project on soil tillage experiment taken up during 2015-20. Surface soil samples (0-0.15 m depth) were collected after the second crop harvest season from two tillage systems and mulching treatments and each of the four treatments were replicated thrice in a Randomized Block Design. The treatment details are as: T1- Conventional tillage with porous weed control ground cover mulching (referred as sheet mulching), T2-Conventional tillage without mulching, T3- Minimum tillage with sheet mulching, T4- Minimum tillage without mulching and T5-Control (without tillage and mulching practice). The land was given with initial ploughings, two times to a depth of 0.22 m using tractor drawn disc plough for conventional tillage. In minimum tillage, soil was disturbed manually during times of initial ridge formation and two times during earthing up operations. Control refers to cultivable lands wherein no tillage and mulching practices performed.

The soil of the experimental site is typical laterite with strongly acidic pH (3.8-4.6). The basic soil physical constants viz. bulk density, particle density, total porosity, water holding capacity were determined using Keen Roczkowski box method as described by Piper (1966). Soil pH was determined with 1:2.5 ratio soil: water suspension (w/v) using pH meter (Jackson, 1973). Soil organic carbon (SOC) was determined by dichromate oxidation method (Walkley and Black, 1934). Dehydrogenase enzyme activity was estimated with 2-3-5-Triphenyl tetrazolium chloride (TTC) reduction technique (Casida, 1977). Carbon mineralization was monitored at 24 h, 48 h and 7 days with organic matter decomposition technique in soil through CO_2 evolution by alkali trap method (Zibilske, 1994). Nitrogen mineralization was determined with micro diffusion method (Conway, 1942). Total glomalin (TG) and easily extractable glomalin (EEG) were determined by the Lowry protein assay with bovine serum albumin standard (Wright and Upadhyaya, 1996).

Results were evaluated by statistical analysis. F-test analysis of variance was performed, and significant difference was calculated at 5% level probability. The effects of treatments with control as well as the differences among the treatments are examined to prove the significant differences. Pearson's correlation was performed in order to seek the relationships between each physical and biological soil variables. Statistical analysis was performed by Wasp 2.0 package for windows (ICAR-CCARI, Goa) and with Microsoft Office Excel programs.

Results and Discussion

Soil physico-chemical properties

The effects of treatments on soil physicochemical properties are given in Table 1.

Table 1. Effect of different tillage and mulching treatments on soil parameters

Treatment	Physico-chemical parameters*				
	Bulk density (Mg m^{-3})	Porosity	Water holding capacity	pH	Soil organic carbon (%)
		(%)			
T1	1.41^{bc}	45.6^{a}	41.3^{a}	3.25^{b}	0.54^{b}
T2	1.45^{bc}	38.9^{b}	34.4^{c}	3.86^{a}	0.54^{b}
T3	1.38^{c}	35.5^{bc}	35.7^{bc}	3.85^{a}	0.60^{b}
T4	1.48^{b}	31.6^{c}	38.4^{ab}	3.89^{a}	0.74^{b}
T5	1.58^{a}	38.4^{b}	37.7^{abc}	3.86^{a}	1.37^{a}
CD (P = 0.05)	0.092	6.54	3.81	0.36	0.21
CV (%)	3.5	9.5	5.6	5.3	15.2

*Values followed with different superscript alphabets are significantly different at P = 0.05

Among the physical properties, soil bulk density and porosity were observed to be optimum in tilled soils and higher value (1.58 Mg m^{-3}) is noticed in control (Table 1). This could be the resultant effect of tillage on loosening the soil as reported previously by Agbede (2006). The maximum WHC (41.3%) was recorded in conventional tillage with sheet mulching (T1) followed by minimum tillage without sheet mulching (T4). Bulk density of the soil was decreased in conventional tillage as compared to control due to increased porosity that resulted in higher water holding capacity of the soil. The result supports the findings of Gerhardt (1997) and Kumar et al*.,* (2015).

There is not much significant change in soil pH because of various tillage practices. However, there was a significant decrease in pH of conventionally tilled soils as compared to control. It has been reported that minimum tillage and conventional tillage lowered pH in the 0-5 cm layer compared to no tillage (Chan et al*.,* 1992) or that tillage did not affect pH (Standley et al., 1990).

The soil organic carbon registered the maximum in control (T5). Minimum tillage practices helped in preserving carbon, as evident from the higher values, though not significant, as compared to conventional tillage probably due to reduced soil disturbance and increased additions of plant biomass. Conservation practice showed net positive effect on increase in depth wise soil organic carbon (SOC) and microbial biomass carbon (MBC) both of which can influence chemical, physical and biological properties (Crystal-Ornelas, 2021). It is also suggested that strong link between accumulations of SOC and microbial community traits under conservation tillage practices pave a way to sustainability of agro-ecosystems. The findings of Rahmati et al., (2020) agree with the result, which concluded that minimum and zero tillage significantly improved the SOC as primary indicator of soil quality. Liu et al., (2020) suggested that conservation tillage could be a viable technology to alleviate the deleterious effect of climate change via carbon sequestration and reduction of green-house gas emission from agricultural activities into the atmosphere. Additionally, it enhances the sustainability of the agricultural production system and resilience to external stresses.

Soil biological properties

The content of dehydrogenase enzyme among the tillage treatments ranged from 0.46-1.25 μg TPF $g^{-1}h^{-1}$ (Table 2).

Table 2. Influence of different treatments on soil biological parameters

Treatment	Soil biological parameters*		
	Dehydrogenase activity (μg TPF $g^{-1}h^{-1}$)	Total Glomalin	Easily extractable Glomalin
		(mg g^{-1})	
T1	1.06^{b}	2.64	0.15^{bc}
T2	0.82^{c}	1.94	0.12^{c}
T3	0.75^{c}	2.59	0.15^{bc}
T4	0.46^{d}	2.46	0.17^{ab}
T5	1.25^{a}	2.70	0.20^{a}
CD (P = 0.05)	0.186	NS	0.044
CV (%)	11.8	18.3	15.2

*Values followed with different superscript alphabets are significantly different at P = 0.05

The maximum content of DHA was measured in control whereas considerable variation was observed among the treatments with and without mulching under both tillage practices. It was found that DHA depends on the availability of soil carbon and a positive correlation between DHA and the labile carbon concentration was reported (Wiatrowska et al., 2021). Dehydrogenase indicates the metabolic activity of soil organisms, especially bacteria and fungi in the soil (Chu et al*.,* 2007; Jarvan et al*.,* 2014; Sharma and Mishra, 1992) and reflects the microbial redox system and the oxidative activities

in soil. Abundance of fungi in conservation tillage (CT) as compared to conventional tillage contributed towards high DHA due to vast amounts of soil C which amplified the soil DHA. The enormous amount of soil carbon in CT encourages the expansion of fungi, which increases soil enzyme activity.

The content of total glomalin among the tillage and mulch treatments ranged from 1.94-2.70 mg g^{-1}. The maximum content of TG was registered in control, but no statistical significance had been observed with other treatments. The content of easily extractable glomalin (EEG) among the treatments ranged from 0.11-0.20 mg g^{-1}. The control treatment recorded the maximum value of 0.20 mg g^{-1} and considerable variation was observed among the treatments. The no-tillage system enhanced easily extractable glomalin contents in the soil surface layers that linked to soil aggregation (Bortolini et al., 2021). In the long run, reduced soil disturbance may be beneficial for the growth of fungi. Therefore, reducing the amount of tillage increased soil health by encouraging soil carbon sequestration and aggregate stability through fungal development as well as the glomalin content. The favorable correlation between N mineralization and EEGRSP (Easily Extractable glomalin Related Soil Protein) reported by previous studies also suggests that EEGRSP may be employed as a measure of soil N availability under a no-till system. The favorable effects of minimum tillage on improved SOM, carbon storage may be due to a positive association of glomalin with water stable aggregates which is maintained favorably by zero tillage during the full crop year. The positive glomalin-WSA association that indicates intact fungal hyphal networks is not preserved by conventional tillage, even though it may increase soil glomalin at various times of the cropping year (Wilkes et al., 2021).

Carbon and nitrogen mineralization

The content of carbon mineralization at 24 hours among the tillage treatment ranged from 62.3- 95.3 increased to 48 hours and later decreased (Table 3).

Maximum quantity of mineralized C (as carbon dioxide) was observed in soils under conventional and minimum tillage with sheet mulching (T1, T3 and in T5) probably due to higher content of soil carbon and increased activity of dehydrogenases. More quantity of particulate organic carbon (POC) in minimum disturbed lands may also contribute to the development of macro-aggregates and aggregate stability at 0–10 cm depth, protecting SOC from mineralization and could be attributed to the differences in mineralization potential of minimum tillage as compared to conventional tillage (T1 versus T3). Additional SOC sequestration may be affected by lengthening the conservation tillage period as explained by Kan et al., (2020). The content of carbon mineralization at 48 hours among the tillage treatment ranged from 99-135.7 mg CO_2 100 g^{-1}. Though there is an increased decomposition noticed in the treatments of conventional tillage with sheet mulching and minimum tillage with sheet mulching, it was not statistically significant. The amount of mineralizable carbon in 7 days among the tillage treatment ranged from 58.3-128.3 mg CO_2 100 g^{-1}. The treatment minimum tillage without mulching recorded the maximum value of 128.3 and considerable variation was observed among the treatments. It was reported that C mineralization was more abundant in soils with minimum physical disturbance, associated with species that could produce extracellular polymeric compounds and had metabolic adaptations for withstanding environmental stress. It is important to understand how soil physical disturbance affects microbial communities across climates and intrinsic soil qualities that contribute changes in C mineralization. According to Vazquez *et al.* (2019) conservation tillage techniques with minimum inversion techniques hasten the mineralization of C, enhance soil C content, and boost microbial biomass and activity.

The content of nitrogen mineralization at 24 hours among the tillage treatment ranged from 2.3-3.7 mg N 100 g^{-1}. Mulching reduced the nitrogen mineralization significantly especially in minimum tillage practices

Table 3. Changes in soil C and N mineralization over time in different treatments

Treatment	C mineralization			N mineralization		
	24 h	48 h	7 days	24 h	48 h	7 days
	(mg CO_2 100g^{-1})			(mg N 100g^{-1})		
T1	95.3^{a}	135.7	66.0^{b}	2.3^{c}	1.6	1.0^{b}
T2	66.0^{bc}	110.0	58.7^{b}	2.5^{bc}	1.6	0.9^{b}
T3	80.7^{abc}	113.7	51.3^{b}	2.6^{bc}	1.3	0.9^{b}
T4	62.3^{c}	99.0	128.3^{a}	3.7^{a}	1.5	1.2^{ab}
T5	88.0^{ab}	102.7	110.0^{a}	2.9^{b}	1.6	1.4^{a}
CD (P = 0.05)	23.7	NS	29.7	0.52	NS	0.33
CV (%)	16.6	16.4	16.7	10.3	10.3	16.6

*Values followed with different superscript alphabets are significantly different at P = 0.05

(Table 3). Lingutla et al., (2019) observed that conservation tillage practices significantly improved the microbial parameters, total nitrogen (total N), mineralizable nitrogen (MN), microbial biomass nitrogen (MBN), and microbial biomass carbon (MBC). The content of nitrogen mineralization at 48 hours among the tillage treatment ranged from 1.34-1.63 mg N 100 g^{-1}. Though there is an increased decomposition noted in control and conventional tillage system, no statistical significance was observed. The content of nitrogen mineralization in 7 days among the tillage treatment ranged from 0.93-1.40 mg N 100 g^{-1}. The treatment control recorded the maximum value of 1.40, considerable variation was observed among the treatments. Vazquez et al., (2019) concluded that it is possible to perceive the more significant effect of no tillage net nitrogen mineralization (NNM) than on C mineralization as a decoupling between the C and N mineralization, which may boost the availability of N for crops and reduce the risk of N losses during the fallow season. The positive effects of long-term no-till management on soil nitrogen stocks, nitrogen mineralization, and effective availability of N are demonstrated by the results of Canisares et al., (2021). From the above study, the minimum tillage favored higher status of carbon, nitrogen mineralization and with improved soil properties. The amounts of SOC and total nitrogen retained in the soil have increased due to enhanced residue retention and minimum tillage methods (Salahin et al., 2021).

Correlation among soil parameters influencing C and N mineralization

Carbon and nitrogen mineralization at 24 hours were significantly correlated ($t=2.28^{*}$; $P=0.05$) with each other. Soil water holding capacity is found to be the major variable associated with the mineralization of C (3.59^{*}). Similarly, nitrogen mineralization at 7 days was found to be significantly correlated with soil organic carbon (4.06^{*}) and EEG (3.20^{*}). Among the soil properties, a positive correlation was found among SOC with bulk density (3.71^{*}) and EEG (3.54^{*}), TG and EEG (4.8^{*}), DHA activity with bulk density (2.52^{*}) and total glomalin with water holding capacity (2.34^{*}). The association of EEG with soil properties indicated the favorable relationship among soil organic carbon, soil dehydrogenases and nitrogen mineralization at 7 days of incubation.

Conclusion

The metabolic activity as measured by dehydrogenase content influenced the carbon mineralization at 24 h and 7 days. There was no significant influence of tillage and mulching practices on total glomalin content. Soils where no tillage practices performed were found to have high EEG content as compared to conventional and minimum tillage practices. Based on the results obtained from the study, it can be inferred that conventional tillage and porous weed control plastic mulch with a thickness of 120 gsm treated soils can be an ideal practice in Ultisols in view of optimum water holding capacity and its effect on soil carbon mineralization.

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Evaluation of improved varieties of cassava in the tribal belts of Attappadi in Kerala

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Abstract

Cassava is an important source of energy in the diet of the people of tropical countries in the world. It has enormous potential in India for food security and industrial uses due to its ability to grow in marginal and waste lands where other crops do not survive. Commercial planting of cassava is done from stem cuttings. Because of the low multiplication rate as compared to cereals and pulses, the high yielding varieties released in the research institute takes many years to reach the farmers. Over the years, clonal multiplication degenerates the planting material, reduce tuber yield drastically, and renders the cultivation of cassava uneconomical. An attempt was made to see the performance of improved varieties of cassava in Pudur gram Panchayat of Attappadi tribal region in Palakkad district which falls under Attappadi Hills laterites Agro Ecological Unit 18 (AEU18) of Kerala. The programme was implemented under the project on Development of Tuber Crops financed by Government of Kerala during 2014-15 and 2015-16 undertaken by ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala. Fifty farmers were selected and quality planting materials of improved varieties of cassava from ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI), Thiruvananthapuram, Kerala were distributed for cultivation in an area of 25 cents of each, with a total areaof 5 ha. The cultivation of cassava was carried out under rainfed conditions with the guidance and the direct supervision of ICAR-CTCRI scientists. Farmers got an average tuber yield of 3.40 kg to 6.50 kg per plant with an average number of tubers from 6 and 12 per plant in Pudur grama panchayat. Improved varieties of cassava produced significantly higher average tuber yield of 59.25t ha^{-1} at Pudur and the farmers also could produce 1.25 lakhs stems of cassava in one season sufficient for cultivation in an area of 80 ha. Adoption of improved varieties was economic with a B:C ratio of 2:1 which could improve their livelihood also, in addition to food security.

Keywords: Cassava, Quality planting material, B:C ratio, Tuber yield

Introduction

Cassava is the fourth most important source of calories in the human diet and has higher carbohydrate content than either maize or rice. Cassava was introduced into India from Brazil by the Portuguese, who landed in the Malabar region of Kerala in the 17th century. Cassava is one of the climate resilient tropical tuber crops used as food, feed and industrial raw material. In India, it is cultivated predominantly in Kerala and Tamil Nadu, and is also grown in Andhra Pradesh, Assam, Karnataka, Madhya Pradesh, Manipur, Nagaland, Tripura, Mizoram and the Andaman & Nicobar Islands. In coastal and

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tribal areas, it is a staple food. It is also the raw material for the starch and sago industry and a component of animal, fish and poultry feeds in many developing nations, including India (Joseph et al., 2004; Yan et al., 2013). In India, cassava is cultivated in an area of 0.183 million ha with a production of 6.94 million tonnes and the productivity in India is the highest (37.93 t ha^{-1}) globally (FAOSTAT, 2021). Tuber crops are rich sources of starch and hence apart from being a source of food, they find an important place in industrial sector as well. Root and tuber crops, most of them being vegetatively propagated,are inherently more prone to the incidence, continuity and dissemination of both systemic and non-systemic diseases than the sexually reproduced crops using true seed as planting material. Reduced availability of quality planting materials is a major hindrance in the faster spread of high yielding varieties and their adoption by the farming community.

Tuber crops are considered as insurance crops during the days of famine or natural calamity. It is an important source of energy for the millions of people in the tropical and subtropical parts of the world (Yan et al., 2013). It produces more calories per unit area per unit time than any other crop. Cassava is mainly grown for its starchy tubers of edible and commercial value. It is an important source of starch and a component of animal, fish and poultry feeds (Abraham et al., 2006; George et al., 2011). High yielding varieties released by research stations take several years to reach the end user because of the extremely low multiplication rate in tuber crops multiplication ratio in cassava to 1:10. Difficulty in transporting the planting materials to distant places due to bulkiness of planting material and the cassava mosaic disease infection are other major hindrancesto the spread of these crop in non-traditional areas of the country. Cassava, with its versatility to adapt to varying soil, climate and edaphic conditions, stand out as unique to meet the food and fuel requirements of ever-increasing population. The ability to yield reasonably well under changing climatic conditions makes it a future crop. The present study was carried out with the objective of assessing the performance of improved varieties of cassavafrom ICAR-Central Tuber Crops Research Institute *viz*., Sree Jaya, Sree Vijaya, Vellayani Hrashwa, Aniyoor, CTM-806, CTM-820, CTM-818 and CTM-815 in Palakkad district of Kerala in tribal belts of Attappadi region of Kerala along with the multiplication of planting materials of the same.

Material and Methods

Performances of improved varieties of cassava were studied at Pudur grama panchayat, Attappadi tribal region of Palakkad district under Attappady Hills Agro ecological unit 18 (AEU18) of Kerala. In this AEU area, the climate is tropical humid monsoon type to sub humid semi arid with a mean annual temperature of 24.3 to 27.6°C and rainfall ranging from 856 to 1482 mm. The soils of Attappadi hills are fertile, and near neutral to slightly alkaline clay soils rich in organic matter. The programme was implemented through Pudur Krishi Bhavan of Palakkad district of Kerala during 2014-15 and 2015-16 under the project on Development of Tuber Crops in the state of Kerala which was undertaken by ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram, Kerala funded by the Department of Agriculture, Government of Kerala. The quality planting materials of eight improved varieties of cassava *viz*., Sree Jaya, Sree Vijaya, Vellayani Hrashwa, Aniyoor, CT- 806, CTM-820, CTM-818 and CTM-815 were distributed to the farmers for cultivation in an area of 25 cents per each beneficiary, and thus covering a total area of 5 ha. Five skill-based training programmes were conducted for fifty farmers on various topics, such as scientific cultivation practices, improved varieties, agro techniques, organic cultivation, plant protection measures, minisett quality planting material production (James George et al., 2004), seed treatment, value addition and seed certification standards. The experts of ICAR-CTCRI, Thiruvananthapuram visited the farmers field periodically and gave technical advice on intercultural operations, remedial measures for incidence of pest and disease, rouging of off types and disease infected plants. The yield data were recorded at the time of harvesting.

Result and Discussion

Based on random sampling, an average cassava tuber yield of 59.26 t ha^{-1} was recorded at Pudur area. The tuber yield varied from 3.20 to 6.50 kg per plant with an average of 4.80 kg per plant. Number of tubers ranged from 6 to 17 per plant with an average of 9.90 per plant (Table1 & Fig.1). The average tuber yield and planting material production per unit area of 25 cents were found to be 6,000 kg and 2500 stems, respectively.

In general, performance of improved varieties of cassava was better in AEU 18. Among the varieties, Sree Vijaya performed better in terms of tuber yield (7.5 kg $plant^{-1}$) and number of tubers (16 $plant^{-1}$) in Chavadiyoor village, followed by Vellayani Hrashwa (tuber yield - 6.4 kg $plant^{-1}$ and number of tuber - 15 $plant^{-1}$in Thekkuvatta village), Aniyoor (tuber yield - 5.8 kg $plant^{-1}$ and number of tuber -10 per plant in Chavadiyoor village). Among the different varieties, lower yield was recorded by Sree Jaya (3.20 kg $plant^{-1}$) in Thekkuvattu village. Earlier report revealed that the temperature variation prevailed during the cultivation might be the major reason for the lesser yield (Muthuraj et al., 2021). Taking into consideration, the cost of cassava stems, transportation,field preparation, planting and other cultivation expenses, total cost of cultivation was estimated as ₹ 20,000 for unit area of 25 cents. The planting materials and cultivations expenses

Table 1. Yield performance of cassava varieties in the farmer field at Pudur Panchayat of Attappadi region, Palakkad

Sl.No.	Name of the farmer	Name of the village	No of tubers plant^{-1}	Tuber yield (kg plant^{-1})	Variety
1.	Mr. C.Kaliappan	Chavadiyoor	10	5.300	Sree Vijaya
2.	Mr. R.Rajappan	Chavadiyoor	16	7.500	Sree Vijaya
3.	Mrs. Vadugi	Palagaiyoor	13	5.000	Vellayani Hrashwa
4.	Mr. T.Rangan	Chirakadavu	10	5.800	Aniyoor
5.	Mr. Kanagaraj	Chavadiyoor	13	5.900	Sree Vijaya
6.	Mr. K.Ravi	Chavadiyoor	11	3.500	Aniyoor
7.	Mr. R.Palanisamy	Thanchapadi	7	3.500	CTM-907
8.	Mrs. Mallika	Palagaiyoor	9	4.500	Sree Vijaya
9.	Mr. K.Eswaran	Padavayal	7	6.000	CTM-907
10.	Mr. G.Selvaraj	Palagaiyoor	7	4.400	CTM-907
11.	Mrs. Umamaheshwari	Chitra Kattavu	10	4.000	CTM-909
12.	P.Krishnakumar	Thekkuvatta	15	6.400	Vellayani Hrashwa
13.	M. Selvan	Chirakaduvu	12	6.500	Sree Vijaya
14.	Mr. Ponnan	Thekkuvatta	6	3.200	Sree Jaya
15.	Mr. Pradeep Kumar	Padavayal	10	5.500	CTM-817
16.	Mr. Sasikumar	Padavayal	12	5.100	Sree Vijaya
17.	Mr. Choriyan	Gottiyarkandi	11	4.800	Sree Jaya
18.	Mrs. Maheswari	Kolappadi	10	4.000	CTM-907
19.	Mr. Veeran	Melapalagaiyur	9	5.500	Sree Vijaya
20.	Mrs. Lakshmi	Vettiyoor	8	4.300	Sree Vijaya
		Total	198	96.000	
		Mean	9.90	4.800	

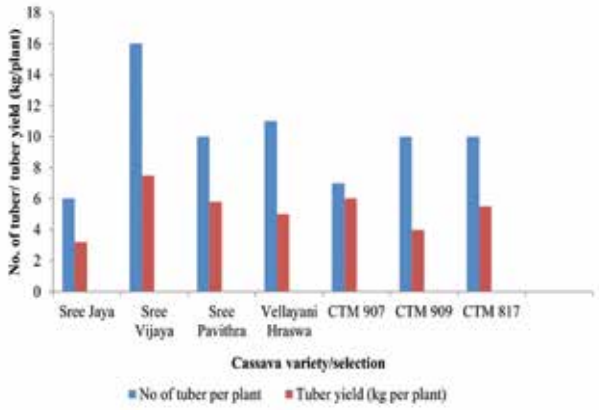

Fig. 1. Tuber number and tuber yield from different cassava varieties/selections at Pudur panchayat

were distributed to beneficiary farmers under the Development of tuber crops scheme. On an average, farmers got a tuber yield of 4.80 kg per plant at Pudur panchayat and 1.50 lakhs of cassava stems of improved varieties could be produced from 5 ha in one season. The planting materials produced under this programme were distributed to the neighbourhood farmers for cultivation in an area of 80 ha.

Table 2. Economics of cassava cultivation in the farmers field at Attappadi (0.25 acre)

Sl. No.	Item	
1	Cultivation expenses including cost of planting material (₹)	20,000
2	Tuber Yieldkg (0.25 acre)	6.0 t
3	Cross returns 20 per kg (₹)	60,000
4	Net return@ (₹)	40,000
5	B:C Ratio	2:1

Conclusion

The eight improved varieties of cassava from ICAR-CTCRI were given to tribal farmers in Attappadi in Palakkad district of Kerala and the crop was raised based on the ICAR-CTCRI package of practices on which training was given to the farmers.The results revealed that the average tuber yield was higher in Pudur (59.26 t ha^{-1}) as compared to local varieties (15.75 t ha^{-1}). Average tuber number was 9.9 plant^{-1} and average tuber yield was 4.80 kg per plant^{-1}. Accordingly, the net income and benefit-cost ratio from cassava crop from an area of 25 cents were ₹ 40,000 and 2:1 respectively was 2:1 in the

Pudur panchayat of Palakkad district of Kerala. Due to implementation of this programme, farmers of Pudur gram panchayat got sufficient good quality planting material of cassava available at the time of planting, and available locally. This helped to cover more area in Attappadi region with improved varieties of cassava and is fast spreading in the neighbourhood area and increased the income generation to farmers.

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Data generation for smart hydroponic system development in *Syngonium podophyllum* (Arrowhead vine)

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Abstract

Hydroponics, a young budding science which can be used as an alternative sustainable production system under limited resource availability conditions. High quality ornamental plants can be produced through hydroponics as it encourages faster growth. Arrowhead vine (*Syngonium podophyllum)* is a widely cultivated ornamental foliage plant as it eliminates polluting agents from inside households. An experiment on standardization of solution culture in Deep Flow Technique (DFT) of hydroponics in *Syngonium podophyllum* was carried out at College of Agriculture, Vellayani, Thiruvananthapuram during the year 2022. The experiment was laid out in completely randomized design. Four doses of Hoagland solution and cooper solution (50%, 100%, 150% and 200%) were given and replicated ten times. Data on growth parameters for development of a smart hydroponic system was collected in scheduled intervals. The electrical conductivity (EC) and pH were monitored at weekly intervals and observations on growth parameters were taken at every 15 days interval for four months. The treatment with 150% Hoagland solution in *Syngonium podophyllum* plants showed superior characteristics for number of leaves, leaf length, leaf breadth, plant height and plant spread. The data can be used for developing an automatic hydroponic system controlled by a mobile application and different ornamental foliage plants can be tested using it.

Keywords: Hydroponics, DFT, *Syngonium podophyllum,* Smart hydroponic system

Introduction

Hydroponics, a young budding science can be used as an alternative sustainable production system under limited resource availability conditions. The per capita availability of living space is reducing due to rapid increase in population explosion and become a major issue in urban communities. Selection of plants grown under indoor environments with limited space is gaining much importance now a days. High quality ornamental plants can be produced through hydroponics as it encourages faster growth (Lakhiar et al., 2018). *Syngonium podophyllum* is a widely cultivated ornamental foliage plant. It has a tremendous ornamental value as it eliminates polluting agents from inside households. It prefers low light conditions to grow hence it is very suitable for indoor gardening. In tropical countries, it is a plant with high export potential. Smart farming is seen to be the future of agriculture as production of high quality crops and intelligent sensing of the controlling parameters can be done efficiently. Development of sensor-based automation is emerging now a days with the help of mobile applications. It is a cheaper and more affordable way of developing small scale hydroponic systems (Modu et al*.,* 2020). The present paper discusses the results of a preliminary study conducted for data generation with the aim of developing a smart hydroponic

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system for *Syngonium podophyllum* controlled by a mobile application.

Materials and Methods

The present experiment was carried out at College of Agriculture, Vellayani, Thiruvananthapuram, Kerala during the year 2022. The experiment was laid out in completely randomized design. Four doses of Hoagland solution (Hoagland and Arnon, 1950) and cooper solution (Lakhiar et al., 2018)(50%, 100%, 150% and 200%) were given so that totally there were eight different treatments in the experiment and each treatment combinations replicated ten times. Generally, pH of the nutrient solution needs to be maintained between 5.5-6.5 (Tellez et al., 2007) and electrical conductivity (EC) between 1.5-2.5 (Sonneveld and Voogt, 2009).The pH and EC in the present study were monitored in all the nutrient solutions periodically. *Syngonium podophyllum*var. 'White Butterfly' was chosen for the experiment. The experiment unit was designed in PVC pipe closed at both ends. Plants were grown in 3 inch net pots.Observations on various growth parameters *viz.,* number of leaves, leaf length, leaf breadth, plant height and plant spread were observed every 15 days interval from 15th day after planting till 120 days after planting. Details about the treatments given are mentioned below.

Solutions

S_1 – Hoagland Solution

S_2 – Cooper's Solution

Doses

D_1 – 50% dose

D_2 – 100% dose

D_3- 150% dose

D_4- 200% dose

Treatment combinations

T_1 : S_1D_1

T_2 : S_1D_2

T_3 : S_1D_3

T_4 : S_1D_4

T_5 : S_2D_1

T_6 : S_2D_2

T_7 : S_2D_3

T_8 : S_2D_4

Results and Discussion

The study on the effect of nutrient solutions on the number of leaves of *Syngonium podophyllum* var. White Butterfly showed that there was a significant impact throughout the crop period. Hoagland solution was found superior to Cooper's solution (Table 1). The highest value was observed at 120 DAP (18.18). Significant effects of various nutrient doses on the number of leaves were observed from 15 DAP to 120 DAP. The 100% nutrient dose was found to be superior from 15 DAP to 45 DAP but that was found to be on par with all other nutrient doses. Superior values for number of leaves were observed for 150% dose of nutrients from 60 DAP to 120 DAP and highest number of leaves were observed at 120 DAP (17.25) which was found to be on par with 200% nutrient dose (16.05). The interaction effect of nutrient solutions and its doses were significant throughout the experiment (Table 2). A significantly higher number of leaves was observed at 120 DAP (24.70) for the plants grown in Hoagland solution applied at 150% dose. This might be due to the higher nutrient availability to plants when applied with 150% Hoagland solution application (Spehia et al., 2018).

Table 1. Main effect of nutrient solutions and its various doses on number of leaves of *Syngonium podophyllum* var. White Butterfly grown under DFT system of hydroponics

Treatment	Days after planting (DAP)							
	15	30	45	60	75	90	105	120
Solution (S)								
S_1	4.975	7.1	8.825	11.800	13.475	14.875	16.70	18.18
S_2	4.175	6.3	7.050	8.225	9.300	9.975	10.65	11.18
CD (0.05)	0.516	0.794	0.906	1.095	1.162	1.181	1.207	1.09
SEm(±)	0.183	0.282	0.322	0.388	0.412	0.419	0.428	0.39
Dose (D)								
D_1	4.65	6.25	7.30	8.60	9.80	10.65	11.65	12.40
D_2	4.90	7.30	8.35	9.55	10.80	11.40	12.15	13.00
D_3	4.65	6.75	7.85	11.80	13.35	14.70	16.10	17.25
D_4	4.10	6.50	8.25	10.10	11.60	12.95	14.80	16.05
CD (0.05)	1.315	1.398	1.425	1.549	1.644	1.671	1.706	1.542
SEm (±)	0.259	0.398	0.455	0.549	0.583	0.593	0.605	0.547

Table 2. Interaction effect of nutrient solutions and its various doses on number of leaves of *Syngonium podophyllum* var. White Butterfly grown under DFT system of hydroponics

Treatment	Days after planting (DAP)							
	15	30	45	60	75	90	105	120
S1D1	4.7	5.8	6.9	8.1	8.4	9.4	10.2	11.0
S1D2	5.4	7.6	8.8	9.8	11.5	12.7	13.3	14.4
S1D3	5.2	8.0	9.9	16.4	18.8	20.3	22.9	24.70
S1D4	4.6	7.0	9.7	12.9	15.2	17.1	20.4	22.6
S2D1	4.6	6.7	7.7	9.1	11.2	11.9	13.1	13.8
S2D2	4.4	7.0	7.9	9.3	10.1	10.1	11.0	11.6
S2D3	4.1	5.5	5.8	7.2	7.9	9.1	9.3	9.8
S2D4	3.6	6.0	6.8	7.3	8.0	8.8	9.2	9.5
CD (0.05)	1.030	1.589	1.813	2.19	2.325	2.363	2.413	2.181
SEm (±)	0.366	0.563	0.643	0.777	0.825	0.838	0.856	0.774

The effect of nutrient solutions on leaf length of *Syngonium podophyllum* was significant throughout the experiment with highest value at 120 DAP (24.90 cm) for Hoagland solution (Table 3). The nutrient doses were also significant on leaf length with highest value for 100% dose throughout the experiment. The highest among it was after 120 DAP (23.62 cm). The interaction effect of nutrient solutions and its doses was significant, but the highest value varied in each observation. This might be due to the significant increase in growth when different nutrient solution doses were applied as stated by Maruo et al., (2002). Significantly higher leaf length was observed 120 DAP (28.62 cm) for plants grown in Hoagland solution with 200% nutrient dose and it was on par with Hoagland solution of 150% dose (27.90 cm) application.

Effect of nutrient solutions on leaf breadth was significant throughout the experiment and found to have highest value at 120 DAP (23.47 cm) for Hoagland solution (Table 4). The effect of nutrient dose on leaf breadth was significant throughout the experiment and significantly higher values were observed for100% nutrient dose

Table 3. Effect of nutrient solutions and its various doses on leaf length (cm) of *Syngonium podophyllum* var. White Butterfly grown under DFT system of hydroponics

Treatment	Days after planting (DAP)							
	15	30	45	60	75	90	105	120
Solution (S)								
S_1	14.16	16.13	18.65	20.01	21.38	22.36	23.87	24.90
S_2	13.94	15.11	16.05	16.85	17.96	18.40	18.99	19.39
CD (0.05)	0.685	0.994	0.718	0.739	0.68	0.67	0.631	0.624
SEm (±)	0.35	0.353	0.255	0.262	0.241	0.238	0.224	0.221
Dose (D)								
D1	13.18	14.56	16.13	17.16	18.48	19.44	20.20	20.77
D2	16.14	17.89	19.44	20.25	21.42	22.22	23.11	23.62
D3	13.35	14.99	16.89	17.78	19.05	19.44	20.95	21.67
D4	13.52	15.02	16.95	18.53	19.73	20.44	21.47	22.54
CD	1.397	1.406	1.015	1.045	0.962	0.947	0.893	0.882
SEm (±)	0.495	0.499	0.36	0.371	0.341	0.336	0.317	0.313
S×D								
S1D1	11.64	12.57	13.64	14.55	15.07	16.42	17.46	18.00
S1D2	15.55	17.94	20.24	20.94	21.81	23.10	24.49	25.08
S1D3	15.58	17.83	20.99	21.97	24.06	24.28	26.83	27.90
S1D4	13.86	16.16	19.73	22.58	24.58	25.65	26.68	28.62
S2D1	14.72	16.54	18.61	19.76	21.88	22.46	22.94	23.54
S2D2	16.73	17.84	18.64	19.56	21.02	21.33	21.72	22.15
S2D3	11.12	12.16	12.79	13.58	14.04	14.59	15.07	15.43
S2D4	13.17	13.88	14.17	14.48	14.89	15.22	16.26	16.45
CD	1.975	1.988	1.435	1.477	1.36	1.34	1.263	1.248
SEm (±)	0.701	0.705	0.509	0.524	0.482	0.475	0.448	0.443

Table 4. Effect of nutrient solutions and its various doses on leaf breadth (cm) of *Syngonium podophyllum* var. White Butterfly grown under DFT system of hydroponics

Treatment	Days after planting (DAP)							
	15	30	45	60	75	90	105	120
Solution (S)								
S_1	14.158	16.125	18.650	20.010	21.380	22.363	23.865	24.900
S_2	13.935	15.105	16.052	16.845	17.958	18.400	18.997	19.392
CD (0.05)	0.786	0.994	0.718	0.739	0.68	0.67	0.631	0.624
SEm (±)	0.35	0.353	0.255	0.262	0.241	0.238	0.224	0.221
Dose (D)								
D1	13.180	14.555	16.125	17.155	18.475	19.440	20.200	20.770
D2	16.140	17.890	19.440	20.250	21.415	22.215	23.105	23.615
D3	13.350	14.995	16.890	17.775	19.050	19.435	20.950	21.665
D4	13.515	15.020	16.950	18.530	19.735	20.435	21.470	22.535
CD	1.397	1.406	1.015	1.045	0.962	0.947	0.893	0.882
SEm (±)	0.495	0.499	0.36	0.371	0.341	0.336	0.317	0.313
S×D								
S1D1	7.06	7.45	8.32	8.74	8.93	9.16	9.37	9.76
S1D2	9.56	10.21	10.54	10.81	11.01	11.65	12.37	12.67
S1D3	9.18	10.55	11.69	11.95	12.22	13.50	16.01	16.54
S1D4	8.61	9.83	10.44	12.24	12.60	14.22	14.39	14.90
S2D1	9.23	10.27	10.45	11.64	12.11	12.84	13.05	13.35
S2D2	10.44	11.51	11.61	11.71	11.85	12.99	13.14	13.36
S2D3	6.99	7.65	7.99	8.06	8.24	9.15	9.39	9.66
S2D4	7.61	7.94	8.17	8.46	8.55	9.88	10.26	10.60
CD	0.949	0.878	0.943	0.911	0.841	0.976	1.375	1.382
SEm (±)	0.336	0.311	0.334	0.323	0.298	0.346	0.488	0.49

from 15 DAP to 105 DAP. But significantly highest value was observed for 150% dose at 120 DAP (13.10 cm) and which was on par with 100% nutrient dose (13.02 cm). The interaction effect of nutrient solutions and nutrient doses were also found significant throughout the experiment. Significantly higher leaf breadth was observed for plants grown in Hoagland solution of 150% nutrient dose (16.54 cm) at 120 DAP. This result was in conformity with the findings of Kang and Iersel (2004).

The effect of nutrient solutions on plant height was significant throughout the experiment and found to have highest value at 120 DAP (35.22 cm) for Hoagland solution (Table 5). The effect of nutrient dose on plant height was significant throughout the experiment and significantly higher values were observed for100% nutrient dose from 15 DAP to 105 DAP. But significantly highest value was observed for 150% dose at 120 DAP (31.91 cm). The interaction effect of nutrient solutions and nutrient doses on plant height was also found significant throughout the experiment. Significantly higher value was observed for Hoagland solution at 150% nutrient dose (43.99 cm) at 120 DAP. The variation in plant growth showed that not only the nutrient content creates difference but the ratio of different ions in the nutrient solution also influences the plant growth (Li and Cheng, 2014).This might be due to interaction among various ions influencing EC and pH in different nutrient solutions and this can lead to accumulation of plant tissues (Dhanraj, 2020).

Effect of nutrient solutions on plant spread was significant throughout the experiment and was highest for the plants at 120 DAP (56.95 cm) in Hoagland solution. The effect of nutrient dose on plant spread was significant throughout the experiment and significantly higher values were observed for100% nutrient dose from 15 DAP to 105 DAP (Table 6). But significantly higher value was observed for 150% dose at 120 DAP (52.66 cm) and which was on par with 100% nutrient dose (51.26 cm). The interaction effect of nutrient solutions and nutrient doses on plant spreadwas also found significant throughout the experiment. At 150% nutrient dose, significantly higher plant spread (74.03 cm) was observed for plants in Hoagland solution at 120 DAP. Various chemical compositions of nutrient solutions can influence the accumulation of biomass, and this is in accordance with the findings of Li and Cheng (2014).

Table 5. Main effect of nutrient solutions and its various doses on plant height (cm) of *Syngonium podophyllum* var. White Butterfly grown under DFT system of hydroponics

Treatment	Days after planting (DAP)							
	15	30	45	60	75	90	105	120
Solutions (S)								
S1	15.262	18.418	21.105	22.89	26.925	29.490	31.580	35.22
S2	13.020	14.608	16.815	18.42	19.970	21.702	22.452	23.735
CD	1.31	1.196	1.012	1.103	1.246	1.104	0.946	1.37
SEm(±)	0.465	0.424	0.359	0.391	0.442	0.392	0.336	0.486
Dose (D)								
D1	13.195	14.760	16.370	18.710	20.880	23.230	24.460	26.10
D2	16.810	19.470	22.030	24.375	26.545	28.245	29.545	30.79
D3	13.765	16.465	18.865	20.470	22.935	25.745	27.940	31.91
D4	12.795	15.355	18.575	19.065	23.430	25.165	26.120	29.11
CD	1.853	1.691	1.431	1.56	1.762	1.561	1.338	1.938
SEm (±)	0.657	0.6	0.508	0.553	0.625	0.554	0.475	0.687

Table 6. Effect of nutrient solutions and its various doses on plant spread (cm) of *Syngonium podophyllum* var. White Butterfly grown under DFT system of hydroponics

Treatment	Days after planting (DAP)							
	15	30	45	60	75	90	105	120
Solutions (S)								
S1	31.773	34.562	39.270	43.025	45.132	48.522	52.852	56.95
S2	25.542	28.090	31.198	32.945	34.737	36.142	37.525	39.90
CD	1.641	1.73	1.501	1.63	1.566	1.473	1.439	1.772
SEm(±)	0.582	0.613	0.532	0.578	0.556	0.523	0.511	0.629
Dose (D)								
D1	24.955	27.210	30.850	33.060	34.960	36.83	39.080	42.53
D2	35.290	38.620	41.460	42.845	44.840	47.33	49.560	51.26
D3	26.830	29.565	34.670	39.060	41.345	44.13	48.230	52.66
D4	27.555	29.910	33.955	36.975	38.594	41.04	43.885	47.26
CD	2.321	2.446	2.122	2.305	2.215	2.083	2.035	2.506
SEm (±)	0.823	0.868	0.753	0.818	0.786	0.739	0.722	0.889
S×D								
S1D1	21.59	23.03	25.11	26.53	28.300	30.65	32.74	35.46
S1D2	39.11	42.97	43.74	44.51	46.540	49.67	53.41	55.39
S1D3	32.99	36.25	44.98	52.50	55.000	59.85	66.89	74.03
S1D4	33.40	36.00	43.25	48.56	50.690	53.92	58.37	62.93
S2D1	28.32	31.39	36.59	39.59	41.620	43.01	45.42	49.59
S2D2	31.47	34.27	39.18	41.18	43.140	44.99	45.71	47.12
S2D3	20.67	22.88	24.36	25.62	27.690	28.41	29.57	31.32
S2D4	21.71	23.82	24.66	25.39	26.498	28.16	29.40	31.58
CD	3.282	3.459	3.001	3.26	3.132	2.946	2.879	3.544
SEm (±)	1.164	1.227	1.065	1.157	1.111	1.045	1.021	1.257

The present study revealed that the vegetative characters of *Syngonium podophyllum* var. White Butterfly grown under hydroponics were superior in Hoagland solution applied at 150% dose. It was also observed that plant growth was promoted till 150% of Hoagland solution and 100% Cooper's solution, but beyond this optimum concentration, the growth decreased and these results are akin to those of Baiyin et al., (2021). Indoor plant production systems were promoted largely by advancements in nutrient solution technologies.

Conclusion

The vegetative parameters *viz.,* number of leaves, leaf length, leaf breadth, plant height and plant spread were maximum for the plants grown with Hoagland solution at 150% concentration and Cooper's solution was found inferior toHoagland solution. The generated data in this study for growth parameters of *Syngonium podophyllum* var. White Butterfly can be used for developing a plant simulation model and different ornamental foliage plants can be tested in a sensor based automatic hydroponic system controlled by a mobile application.The testing can be extended to other ornamental foliage plants also, including those in Ipomoea genus of family Convolvulaceae most known as morning glories.

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Influence of organic and inorganic fertilizers on dynamics of nitrogen, phosphorus and potassium in relation to yield and proximate composition of Colocasia

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Abstract

Field experiments were conducted during 2018-2020 to study the effect of integrated use of inorganic fertilizers and organic manure on the dynamics of nitrogen, phosphorus and potassium in relation to yield and proximate composition of colocasia (*Colocasia esculenta* L) in an Alfisol. The inorganic fractions and available nutrient contents of N, P and K were found highest due to integrated application of FYM and ½ NPK followed by application of 80-30-80 kg ha^{-1} of N, P and K. Sequence of occurrence of inorganic P fractions (mg kg^{-1}) in the soils followed the order: Reductant soluble P (42.39) > Fe-P (38.10) > Ca-P (30.23) > Al-P (23.71) > Bray's-1-P (17.86) > Water soluble P (2.94). Occurrence of different K fractions (kg ha^{-1}) was in the order: NH_4OAc-K (210.05) > exchangeable-K (186.56) > non-exchangeable K (111.54) > water soluble-K (23.49). Available N was contributed mostly by NO_3-N and total N. All the inorganic P fractions contributed significantly to the available P pool and the relationship (r) was found to be in the order of water soluble P (0.97^{**}) > Fe-P (0.96^{**}) > RS-P (0.95^{**}) > Al-P (0.94^{**}) > Ca-P (0.90^{**}). However, exchangeable K and total K contributed significantly towards the available K content of the soil. Ammoniacal N showed a highly positive and significant relationship with corm yield and biochemical constituents of taro. Iron bound P and Al-P fractions contributed mostly towards the P nutrition of colocasia. Of all the K fractions, non- exchangeable K has recorded higher 'r' values with cormel yield and bio-chemical constituents. Application of organic manure and half of the soil test based NPK not only sustain the soil quality and enhanced the productivity of colocasia but also had greater impact on NPK transformations in Alfisols.

Keywords: Inorganic fertilizers, Organic manure, NPK fractions, Colocasia, Correlations

Introduction

The fertility status of Indian soils has been declining continuously due to intensive cropping and non-restoration of nutrients in the soil. The replenishment of reserves of nutrients are necessary which are removed or lost from the soil for maintaining productivity and sustainability of the farming systems. Deficiency of major nutrients especially nitrogen (N) and potassium (K) is prevailing in the Indian soils resulting in sharp decline in production and productivity of nutrient responsive crops. Nitrogen is the most important nutrient for plant growth, yield and quality as it acts as a key component of amino acids, auxins, cytokinins, alkaloids, glucosinolates and proteins. There are three major forms of nitrogen in the soil- organic N, ammonium N and nitrate N. Organic

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forms of N make up for the highest percentage (>90%) of the total nitrogen in the soil, only a small part is in the inorganic or mineral nitrogen forms. Plants can use only ammonium and nitrate forms. Ammonium nitrogen exists in exchangeable and non-exchangeable forms. Potassium exists in four forms in the soil, which include the solution, exchangeable, nonexchangeable or fixed and mineral or structural K forms (Sparks, 2000). The amount of each K fraction varies, depending on cropping history, as well as application of chemical fertilizer or organic manures (Ioannou et al., 1994; Yawson et al., 2011). There is a dynamic equilibrium among different forms of soil potassium and any depletion in a given form would shift the equilibrium in the direction to replenish it (Ramamoorthy and Velayutham, 1976).

Application of chemical fertilizers alone causes problems not only to the soil health but also to the human health and physical environment. To combat this problem, it is necessary to use organic manures alone or with chemical fertilizers that will not only boost agricultural production but also save the environment. Low organic matter status of the soils combined with imbalanced use of inorganic fertilizers, less use of organic manures and inadequate attention given for its improvement and maintenance led to low crop productivity. Continuous cropping and long-term fertilization are liable to change the soil properties and crop production, depending upon the type of management practices. Long-term fertilizer experiments provide best possible means to study changes in the soil properties, dynamics of nutrients and future strategies for maintaining soil health.

Colocasia (*Colocasia esculenta* L.) is a tropical tuber crop with high yield potential of the corms and cormels, broadly grown as food crop and animal fodder which are widely cultivated in tropical and subtropical regions. It is widely cultivated throughout the West Indies and in West and North Africa with a global production of 12.396 Mt from 1.794 Mha with a productivity of 6.91 t ha^{-1}. In Asia, it is widely planted in south and central China and grown in almost all the states in India. Africa contributed 9.53 Mt and Asia contributed 2.395 Mt of production. In many islands of the Pacific including Papua New Guinea, it plays an important role in traditional gifts given in ceremonies. The corms of taro is relatively low in protein (1.5%), fat (0.2%), fiber (0.8%) and ash (1.2%) and high in starch (70-80 g/100 g dry taro). Taro is also a good source of vitamin B_1, B_2, B_3, B_6, C, and minerals such as iron, phosphorus, zinc, potassium, copper and manganese (Quach et al., 2003). Taro starch is easily digestible, hypoallergenic in nature and also the starch is gluten free (Kochhar, 1998).

To improve the yield and quality of taro, there is a need to standardize the optimum dose of nutrients for better physico-chemical properties of soil. The integrated nutrient management (INM) approach helps to improve the quality of the produce andto improve the soil fertility including the biosphere. The previous studies related to INM and SSNM strategies in tuber crops proved to be beneficial in enhancing the crop productivity and improving the fertility status of soil (Laxminarayana, 2016; Laxminarayana, 2022; Laxminarayana et al., 2015). Very scanty information is available on nutrient fractionation studies under tuber crops based cropping systems. The present study aimed to assess the distribution of inorganic fractions of nitrogen, phosphorus and potassium in relation to the yield and bio-chemical constituents of colocasia in an Alfisol of Eastern India.

Materials and Methods

Field experiments were conducted for two *kharif* (rainy) seasons during 2018-19 and 2019-20 at the Regional Station of ICAR-Central Tuber Crops Research Institute, Bhubaneswar, Odisha, India to study the effect of integrated use of inorganic nutrients and organic manure on dynamics of NPK fractions and residual soil properties in relation to yield and proximate composition of colocasia in an Alfisol. The experiment was laid out with 14 treatment combinations replicated thrice in a randomized block design. The treatments included control, 40 kg N ha^{-1}, 80 kg N ha^{-1}, 120 kg N ha^{-1}, 30 kg P_2O_5 ha^{-1}, 40 kg K_2O ha^{-1}, 80 kg K_2O ha^{-1}, 120 kg K_2O ha^{-1}, 80-30 kg N & P_2O_5 ha^{-1}, 80-80 kg N &K_2O ha^{-1}, 30-80 kg P_2O_5&K_2O ha^{-1}, 80-30-80 kg N, P_2O_5 & K_2O ha^{-1}, Farmyard manure (FYM) @ 10 t ha^{-1}, and FYM @ 10 t ha^{-1} + 40-15-40 kg N, P_2O_5 & K_2O ha^{-1} (½ NPK).

Application of well rotten farmyard manure (FYM) (contained 0.50-0.28-0.60% of N, P & K, respectively) @ 10 t ha^{-1}) supplemented 50, 28 and 60 kg ha^{-1} of N, P and K, respectively. Colocasia (*cv* Muktakeshi) cormels were planted at a spacing of 50 × 30 cm. All the intercultural practices were followed as per the schedule and the crop was harvested at the maturity of 165 days after planting. Yield parameters like number of cormel $splant^{-1}$, average cormel weight, cormel yield per plot were recorded at harvest. Cormel samples were collected at harvest, washed thoroughly, oven dried at 60°C and dry weights were recorded. Total sugars in the fresh cormels after washing were estimated in the alcohol filtrate and starch was determined in the residue as per the standard procedure.

Soil samples at a depth of 0.30 m were collected from individual treatments of the experiment after harvest of the crop, processed and estimated chemical properties by using standard procedures. Inorganic forms of N, namely NH_4-N and NO_3-N were determined by steam distillation method (Black, 1965). Inorganic P fractions

were determined by sequential fractionation method as outlined by Jackson (1973). Inorganic K fractions were determined by boiling nitric acid method (Wood and deTurk, 1941). The relationship of different fractions of N, P and K with yield and proximate composition of colocasia was worked out by computing simple correlation coefficients by employing standard procedure as described by Gomez and Gomez (1984).

Results and Discussion

Effect of organic and inorganic nutrients on soil chemical properties

The pH of the soil after harvest of colocasia for the second kharif season during 2019-20 ranged from 6.188 to 6.326 in comparison to the initial value of 6.094 (Table 1); however, the highest increase of soil pH was observed due to integrated application of FYM + ½ NPK. Application of graded doses of N or K fertilizers alone showed an increasing trend of pH up to 80 kg ha^{-1} and showed a declining trend at higher doses of respective nutrients. Application of K fertilizers showed an increasing trend of soil pH in comparison to N fertilizers, whereas combined application of N & K fertilizers showed relatively higher soil pH rather than single application of N & K. Organic manure can buffer pH and thus counter the soil acidity. Incorporation of FYM has improved the soil pH to 6.24 and FYM in combination with limited doses of NPK has further improved the soil pH to 6.33. The rise of soil pH through addition of FYM could be caused by specific adsorption of organic anions and the corresponding release of hydroxyl ions (Hue, 1992) as well as the consumption of H^+ by the humic type substances which have a large number of carboxyl, and phenolic functional groups (Stevenson, 1994). This agrees with the findings of the present study also. Organic matter has high cation exchange capacity and it facilitated retention of exchangeable bases, which causes improvement of soil pH (Ossom and Rhykerd, 2008).

Integrated application of FYM + ½ NPK has recorded the highest organic C content (0.373%) followed by $N_{80}P_{30}K_{80}$ (0.324%) as compard to the initial status of 0.203%. Continuous cropping without fertilization or manuring of the soil led to reduction in organic C content in the control (0.206%). Addition of inorganic fertilizers along with organic sources led to improvement in organic matter status of the soil, which might be due to the enhanced root growth and production of more crop residues leading to accumulation of more organic matter in the soil (Rani Kumari et al., 2019). Incorporation of FYM alone showed an improvement in organic C (0.248%), emphasizing that apart from yield gains, organic sources add organic matter, improve the soil physical and chemical properties and neutralize the soil acidity (Fageria, 2012). Graded doses of N and K fertilizers showed a significant improvement in organic C status, however, dual application of NK showed relatively

Table 1. Effect of application of organic and inorganic nutrients on soil chemical properties

Treatment	pH (1:2.5)	Org. C (%)	Available nutrient (kg ha^{-1})			Exch. Ca	Exch. Mg	Avail. S (mg kg^{-1})	Available micro nutrient (mg kg^{-1})			
			N	P	K	[c mol (p^+) kg^{-1}]			Fe	Cu	Mn	Zn
Initial	6.094	0.203	200.8	35.17	182.8	24.46	7.562	8.24	10.35	1.43	4.18	0.622
Control	6.233	0.206	155.8	35.19	174.6	21.54	6.79	7.49	8.90	1.37	4.24	0.625
N_{40}	6.202	0.257	166.9	36.80	188.7	24.93	7.17	7.56	10.17	1.39	4.23	0.680
N_{80}	6.225	0.273	175.4	37.61	203.6	26.17	7.58	7.58	10.54	1.43	3.28	0.661
N_{120}	6.188	0.312	193.3	38.12	225.5	26.84	8.27	7.62	10.74	1.42	3.56	0.704
P_{30}	6.272	0.255	160.5	40.97	179.8	24.45	7.26	7.53	10.12	1.40	4.08	0.635
K_{40}	6.210	0.247	162.3	37.48	196.2	25.13	6.77	7.58	10.84	1.51	3.94	0.697
K_{80}	6.262	0.257	170.3	38.28	217.7	26.16	7.61	7.67	11.52	1.50	3.87	0.717
K_{120}	6.233	0.275	189.8	40.95	231.0	25.85	7.47	7.71	10.93	1.52	3.79	0.709
$N_{80}P_{30}$	6.262	0.237	182.2	42.76	213.8	25.96	7.67	7.72	11.23	1.47	3.87	0.636
$N_{80}K_{80}$	6.317	0.287	194.3	40.59	232.2	26.79	8.74	7.79	11.58	1.48	3.76	0.685
$P_{30}K_{80}$	6.307	0.263	180.7	44.24	220.6	25.66	7.81	7.75	11.26	1.41	4.06	0.669
$N_{80}P_{30}K_{80}$	6.291	0.324	220.9	46.17	247.5	27.34	9.06	8.07	11.48	1.46	4.50	0.717
FYM	6.243	0.248	176.1	38.73	191.3	25.83	7.70	7.78	10.37	1.40	4.32	0.674
FYM + $N_{40}P_{15}K_{40}$	6.326	0.373	212.8	46.97	245.7	28.12	9.94	8.10	11.75	1.57	4.59	0.701
CD (P=0.05)	0.065	0.029	9.17	2.26	11.5	0.47	0.29	0.07	0.32	0.07	0.49	0.025

higher organic C (0.287%) rather than PK (0.263%) and NP (0.237%). Organic carbon in the soil acts as energy substrate for proliferating microorganisms and enhancing nutrient availability to the crops (Wu et al., 2020).

Highest built up of exchangeable Ca & Mg [28.12 & 9.94 c mol (p^+) kg^{-1}] as well as available S (8.10 mg kg^{-1}) were recorded due to integrated application of FYM + ½ NPK, which can attributed to the combined application of organic fertilization and limited doses of NPK. The available S in the soils was found lower than the critical limit of 10.0 mg kg^{-1} in all the treatments. Higher buildup of available S was observed in the soil with the combined application of N, P and K rather than single application of graded doses of N and K fertilizers.

The available Fe & Mn contents were found toxic (>9.0 and 4.0 mg kg^{-1}, respectively) in all the treatments, which might be due to the soil forming factors and the nature of parent materials from which the soils are formed. Application of nitrogenous fertilizers have resulted higher accumulation of Fe & Mn rather than K; however, organic manure (FYM) showed lower accumulation of Fe & Mn, which might be due to the countering of soil acidity. The available Cu content in the post harvested soils was high (>0.40 mg kg^{-1}), whereas the available Zn content in the soils of the present study was medium (0.60-1.20 mg kg^{-1}). Incorporation of organic sources with inorganic chemical fertilizers showed lower available Fe & Mn and higher contents of available Zn than those of inorganic fertilized plots. The available Fe, Cu, Mn and Zn contents in the post harvest soils were found higher than the critical limits that was attributed to the nature of parent materials and other soil forming factors (Anderson, 1988).

Effect of organic and inorganic fertilization on inorganic nitrogen fractions

Total N in the soils was in the range of 1745.5 to 1986.8 kg ha^{-1} and that of the initial soil was1791.3 kg ha^{-1} (Table 2). The highest total N (1986.8 kg ha^{-1}) was observed in the integrated application of FYM combined with ½ NPK followed by 80-30-80 kg ha^{-1} of N, P_2O_5 and K_2O (1923.2 kg ha^{-1}). This could be attributed to N mineralization pattern of these organics and indirect influence on physico-chemical characteristics of the soil (Singh et al., 2002). Total N was significantly increased with the graded doses of both N and K up to120 kg ha^{-1}. Dual application of $N_{80}K_{80}$ showed relatively higher total N than that of $N_{80}P_{30}$ and $P_{30}K_{80}$. Long term application of organic manure combined with NPK fertilizers increased the contents of soil organic matter, N, P and K, as well as the enzyme activities. The humus produced from the organics on their decomposition, can supply the essential nutrients slowly but steadily to the growing crops besides direct supply from the inorganic fertilizers that contributed in improvement of available nutrient status of the soil (Magdoff and Harold Van Es, 2021).

Table 2. Effect of organic and inorganic nutrients on distribution of inorganic fractions of nitrogen, phosphorus and potassium under colocasia cropping system

Treatment	Nitrogen fractions (kg ha^{-1})				Phosphorus fractions (mg kg^{-1})							Potassium fractions (mg kg^{-1})				
	Total N	Avail. N	NH_4-N	NO_3-N	Total P	Avail. P	WS-P	Fe-P	Al-P	Ca-P	RS-P	Total K	Avail. K	WS-K	Exch. K	Non exch. K
Initial	1791.3	200.8	74.65	16.71	204.56	15.70	2.41	32.65	20.58	28.67	40.170	2153.2	182.8	20.44	162.38	106.24
Control	1745.5	155.8	72.78	15.84	182.47	15.71	2.35	28.22	19.6	28.09	36.650	2072.5	174.6	17.45	157.16	79.97
N_{40}	1790.8	166.9	84.72	18.29	197.69	16.43	2.63	34.78	20.36	27.8	39.420	2130.3	188.7	18.19	170.50	92.36
N_{80}	1845.0	175.3	102.23	20.57	205.29	16.79	2.87	35.26	22.39	28.16	40.260	2214.7	203.6	20.61	182.96	104.80
N_{120}	1864.9	193.3	115.64	22.68	204.78	17.02	2.82	35.89	21.74	27.98	42.720	2295.0	225.5	22.68	202.80	111.30
P_{30}	1755.2	160.5	80.76	17.12	219.32	18.29	3.15	38.92	24.82	30.8	44.580	2115.4	179.8	18.60	161.16	84.80
K_{40}	1775.3	162.3	82.98	17.65	199.58	16.73	2.74	35.15	22.57	26.54	38.190	2209.1	196.2	22.19	174.03	104.20
K_{80}	1804.4	170.3	92.46	18.36	204.63	17.09	2.81	35.94	23.84	27.3	39.980	2324.5	217.7	25.12	192.53	116.72
K_{120}	1839.8	189.8	104.13	18.83	206.32	18.28	2.95	37.12	24.12	28.19	41.850	2394.8	231.0	29.84	201.14	128.80
$N_{80}P_{30}$	1856.3	182.2	104.98	20.68	208.91	19.09	3.23	41.72	25.72	32.09	45.110	2250.3	213.8	21.32	192.47	108.90
$N_{80}K_{80}$	1892.7	194.3	110.45	22.14	205.88	18.12	3.08	39.69	25.86	29.84	42.060	2364.7	232.2	27.80	204.38	126.82
$P_{30}K_{80}$	1825.6	180.7	93.55	19.36	213.81	19.75	3.16	43.38	25.49	34.16	46.390	2349.6	220.6	26.04	194.53	124.84
$N_{80}P_{30}K_{80}$	1923.2	220.9	117.69	23.91	215.67	20.61	3.52	45.66	27.05	35.78	48.500	2470.2	247.5	30.52	216.94	138.60
FYM @ 10 t ha^{-1}	1856.7	176.1	92.87	17.29	206.19	17.29	2.74	38.10	23.42	30.15	40.940	2204.3	191.3	22.16	169.09	110.60
FYM + $N_{40}P_{15}K_{40}$	1986.8	212.8	120.58	24.93	217.08	20.97	3.58	49.06	28.16	37.94	49.060	2453.6	245.7	29.36	216.30	134.20
Mean	1836.9	182.8	96.70	19.62	206.15	17.86	2.94	38.10	23.71	30.23	42.39	2266.8	210.1	23.49	186.56	111.54
Em (+/-)	17.64	5.12	3.98	0.73	2.49	0.43	0.09	1.40	0.66	0.92	1.00	33.30	6.28	1.19	5.21	4.78

The available nitrogen ($KMnO_4$-N) status of the soil ranged from 155.8 to 220.9 kg ha^{-1} with the highest value observed for the balanced application of 80-30-80 kg ha^{-1} of N, P and K followed by FYM + ½ NPK (212.8 kg ha^{-1}). Addition of nitrogenous fertilizers tends to increase the available N status of the soil by 7.1, 12.6 and 24.1% with respect to 40, 80 and 120 kg N ha^{-1} respectively over the control. Dual application of $N_{80}K_{80}$ showed relatively higher available N (194.3 kg ha^{-1}) than application of $N_{80}P_{30}$ and $P_{30}K_{80}$. The available N was found deficient (<250 kg ha^{-1}) in all the treatments. Higher availability of N could be due to the integrated application of mineral fertilizer N along with organic sources which have contributed to the reduction of C:N ratio and thus increased the rate of decomposition resulting in faster availability of nutrients from manures (Varalakshmi et al., 2005).

The range of ammoniacal N and nitrate-N in the soil varied from 72.78 to 120.58 and 15.84 to 24.93 kg ha^{-1}, respectively with the highest contents obtained for integrated application of FYM + ½ NPK. Addition of nitrogenous fertilizers tends to increase the NH_4-N status of the soil by 16.4, 40.5 and 58.8% with respect to 40, 80 and 120 kg ha^{-1} over the control. Addition of nitrogenous fertilizers enhanced theNO_3-N status of the soil by 15.5, 29.9 and 43.2%with respect to 40, 80 and 120 kg ha^{-1} over the control. Dual application of N, P and K fertilizers improved the NO_3-N status in comparison to application of single nutrients. It was also confirmed that the level of both NH_4^+- N and NO_3^--N increased with the increased level of fertilizer N, which was also reported by Duraisami et al., (2001).

Effect of organic and inorganic fertilization on inorganic phosphorus fractions

The total phosphorus in the soil varied from 182.4 to 217.1 mg kg^{-1} whereas the initial content was 204.6 mg kg^{-1} (Table 2). The highest total P content was recorded in the treatment of integrated application of FYM + ½ NPK (217.1 mg kg^{-1}). Combined application of N, P and K fertilizers enhanced the total P status in comparison to application of single nutrients. The available P (Bray's-1-P) in the soils ranged from 15.71 to 20.97 mg kg^{-1} when compared to the initial status of 15.70 mg kg^{-1}. The highest available P was observed in the combined application of FYM + ½ NPK (20.97 mg kg^{-1}). Addition of 30 kg P_2O_5 ha^{-1} increased the available P status of the soil by 16.4% over the control. Dual application of $P_{30}K_{80}$ showed an increase of 25.7% available P followed by $N_{80}K_{80}$ (21.48 %) over the control. Increase in available P content of the soil was attributed to the decomposition of organic manures which could have enhanced the labile P in the soil by complexing Ca, Mg and Al and solubilization of phosphate rich organic compounds through the release of organic acids upon decomposition of organic matter and chelation of organic anions with Fe and Al resulting effective solubilization of inorganic phosphates in the soil (Laxminarayana, 2022).

The sequence of occurrence of inorganic P fractions in the soil was in the order of RS-P > Fe-P > Ca-P > Al-P > Bray's-1-P > Water soluble P. Integrated application of organic manure with the chemical fertilizers enhanced the buildup of all the forms of inorganic-P (Rokima and Prasad, 1991). The water soluble P (WS-P) in the soils varied from 2.35 to 3.58 mg kg^{-1},whereas the initial WS-P

Table 3. Effect of organic and inorganic nutrients on yield and proximate composition of colocasia (2018-2020)

Sl. No.	Treatment	Cormel yield (t ha^{-1})			Yield response (%)	Proximate composition (%)		
		2018-19	2019-20	Mean		Starch	Total sugars	Dry matter
1	Control	10.11	15.34	12.73	-	10.86	0.86	22.56
2	40 kg N ha^{-1}	12.28	22.02	17.15	34.7	11.19	0.93	22.79
3	80 kg N ha^{-1}	13.91	26.02	19.97	56.9	11.53	0.98	23.40
4	120 kg N ha^{-1}	14.05	25.55	19.80	55.5	11.93	1.05	23.61
5	30 kg P_2O_5 ha^{-1}	12.40	20.06	16.23	27.5	11.32	0.94	23.22
6	40 kg K_2O ha^{-1}	12.56	23.24	17.90	40.6	11.57	0.90	23.10
7	80 kg K_2O ha^{-1}	14.10	25.70	19.90	56.3	11.99	1.03	23.61
8	120 kg K_2O ha^{-1}	14.81	26.73	20.77	63.2	12.11	1.11	24.24
9	80 kg N & 30 kg P_2O_5 ha^{-1}	14.27	24.10	19.19	50.7	11.53	1.09	23.76
10	80 kg N & 80 kg K_2O ha^{-1}	17.20	27.10	22.15	74.0	12.39	1.21	24.22
11	30 kg P_2O_5 & 80 kg K_2O ha^{-1}	15.21	25.52	20.36	59.9	11.95	1.11	24.03
12	80-30-80kg N, P_2O_5 & K_2O ha^{-1}	18.46	28.68	23.57	85.2	12.92	1.26	24.67
13	FYM @ 10 t ha^{-1}	13.69	25.04	19.37	52.2	11.61	1.05	23.41
14	FYM @ 10 t ha^{-1} + ½ NPK	19.28	29.26	24.27	90.7	12.87	1.26	24.59
	CD (P=0.05)	0.50	1.10	0.61	--	0.15	0.03	0.12

was 2.41 mg kg^{-1} with the highest value observed for the integrated application of FYM and ½ NPK (3.58 mg kg^{-1}). Most of the WS-P added to the soil is transformed into relatively insoluble inorganic compounds of Al and Fe and thereby reduced its availability for plant use. However, after a time when intensity factor of the soil solution goes down, these inorganic P fractions may contribute to the P nutrition of crops.

The iron bound-P in the soil was in the range of 28.2-46.1 mg kg^{-1} and the initial status was 32.7 mg kg^{-1}. The highest Fe-P content of 46.2 mg $kg^{-1}$was observed in integrated application of FYM + ½ NPK followed by $N_{80}P_{30}K_{80}$ (45.7 mg kg^{-1}). Addition of 30 kg P_2O_5 ha^{-1} resulted in 37.9% increase in the Fe-P content of the soil than the control, however, dual application of $P_{30}K_{80}$ recorded an increase of 53.7% Fe-P. The aluminum bound P in the soils varied from 19.6 to 28.2 mg kg^{-1} when compared to the initial status of 20.6 mg kg^{-1} and the highest value was observed for FYM + ½ NPK (28.2 mg kg^{-1}). Addition of 30 kg of P_2O_5 ha^{-1}caused 26.6% increase in the Al-P content of the soil over the control, whereas dual application of $N_{80}K_{80}$, $N_{80}P_{30}$ and $P_{30}K_{80}$ showed an increase of 31.9, 31.2 and 30.1Al-P respectively over the control.

The initial status of Ca-P in the soil was 28.7 mg kg^{-1} whereas it varied from 26.5 to 37.9 mg kg^{-1}after harvest. The highest Ca-P was observed in the integrated application of FYM+½ NPK (37.9 mg kg^{-1}) followed by $N_{80}P_{30}K_{80}$ (35.8 mg kg^{-1}). Addition of 30 kg P_2O_5 ha^{-1} alone showed an increase of 9.6% and dual application of $P_{30}K_{80}$ and $N_{80}P_{30}$ recorded an increase of 19.1 and 11.9% of Ca-P over the control. The reductant soluble phosphorus (RS-P) in the soils ranged from 36.7 to 49.1 mg kg^{-1}, whereas the initial status was 40.2 mg kg^{-1} and the highest value of RS-P was due to the integrated use of FYM + ½ NPK (49.1 mg kg^{-1}). Addition of 30 kg P_2O_5 ha^{-1} recorded an increase of 21.7% of RS-P, whereas dual application of $P_{30}K_{80}$ and $N_{80}P_{30}$ resulted in a buildup of 26.6 and 17.5% of RS-P over the control, respectively.

Effect of organic and inorganic nutrients on inorganic potassium fractions

Total potassium in the soil ranged from 2072 to 2470 kg ha^{-1} with the highest being recorded for the application of $N_{80}P_{30}K_{80}$ (2470 kg ha^{-1}). Addition of graded doses of K fertilizers showed an increase intotal K status of the soil by 6.6, 12.2 and 15.6% in respect of 40, 80 and 120 kg ha^{-1} over the control. Dual application of $N_{80}K_{80}$ showed relatively higher total K (2364 kg ha^{-1}) than application of $P_{30}K_{80}$ (2350 kg ha^{-1}). The distribution of K forms in the soil and the equilibrium between them determine the K status of the soil and the potential of K supply to the plants (Srinivasa Rao et al., 2000). The available K (NH_4OAc-K) in the soils ranged from 174.6 to 247.5 kg ha^{-1} with the highest for the treatment of balanced application of 80-30-80 kg ha^{-1} of N, P and K (247.46 kg ha^{-1}). The available K (NH_4OAc-K) in the initial soil was182.8 kg ha^{-1}. Addition of potassium fertilizers resulted in an increase the available K status of the soil by 12, 25 and 32% respectively over the control. Dual application of $N_{80}K_{80}$ showed relatively higher available K (232.2 kg ha^{-1}) than other combinations. The crop requirements were partly met from the released K and both the applied K and released K brought out available K build up in the soil. Addition of limited doses of NPK combined with organic manure showed a comparatively marginal increase in available K than that of inorganic and organic sources. The differential release pattern of non-exchangeable K from the soil reserves besides variation in K uptake by the crop would be responsible for such differences in the available K status of the soil (Svotwa et al., 2007).

The highest water soluble potassium (WS-K) was recorded due to balanced application of 80-30-80 kg ha^{-1} of N, P and K (30.52 kg ha^{-1}) followed by FYM + ½ NPK (29.36 kg ha^{-1}). Addition of potassium fertilizers tends to increase the WS-K status of the soil by 27, 44 and 71% in respect of 40, 80 and 120 kg ha^{-1} over the control. Dual application of $N_{80}K_{80}$ showed relatively higher WS-K (27.80 kg ha^{-1}) followed by $P_{30}K_{80}$ (26.04 kg ha^{-1}). Water soluble K in the soil was generally low probably because of the leaching and erosion losses of applied K fertilizers. Organic materials, during their decomposition produce large number of organic acids which might have a tendency to dissolve potassium present either in mineral form or in the non-exchangeable form, thereby bringing it into water soluble form (Mukta Rani et al., 2020).

The exchangeable potassium (Ex. K) of the soil ranged from 157.2 to 216.9 kg ha^{-1} and the initial status was162.4 kg ha^{-1} with the highest being recorded due to the balanced application of $N_{80}P_{30}K_{80}$ (216.9 kg ha^{-1}) at par with FYM + ½ NPK (216.3 kg ha^{-1}). Addition of FYM could increase the CEC of the soil, which can hold more exchangeable K and convert non-exchangeable-K to exchangeable form, consequent to mass action effect (Srinivasa Rao et al., 2002). Addition of potassium fertilizers tends to increase the Ex. K status of the soil by 11, 23 and 28% in respect of 40, 80 and 120 kg ha^{-1} over the control. Dual application of $N_{80}K_{80}$ showed relatively higher Ex. K (204.38 kg ha^{-1}), which was followed by application of $P_{30}K_{80}$ (194.53 kg ha^{-1}). Combined application of N, P and K fertilizers showed greater exchangeable K status than that of individual nutrients. The non-exchangeable K of the soil ranged from 80.0 to 138.6 kg ha^{-1} and that of the initial soil was 106.2 kg ha^{-1}

with the highest being due to the balanced application of $N_{80}P_{30}K_{80}$ (138.6 kg ha^{-1}) followed by FYM + ½ NPK (134.2 kg ha^{-1}). Addition of potassium fertilizers resulted in an increase in non-exchangeable K status of the soil by 30, 46 and 61% in respect of 40, 80 and 120 kg ha^{-1} over the control. Dual application of $N_{80}K_{80}$ showed relatively higher non-Ex. K (126.82 kg ha^{-1}) than $P_{30}K_{80}$ (124.84 kg ha^{-1}). Studies have shown that a significant portion of K (70 - 90%) required by plants comes from the non-exchangeable pool in the absence of easily supplied K, thus indicating the beneficial role of the fixed K (Singh and Singh, 2002). The quantity of interlayer K in vermiculite and illite containing soils showed higher K uptake by the crops. This interlayer K is also the major source controlling the long-term K supplying potential of soils (Escudey et al., 1997). The non-exchangeable K fraction is released when the level of soil solution and Ex. K are decreased by plant uptake and leaching (Martin and Sparks, 1983). Conversion of exchangeable and water soluble K into non-exchangeable forms is a slow process but this equilibrium also plays an important role in K-nutrition of plants as it helps to maintain the non-exchangeable K content of the soils (Dhillon et al., 1985). It was also reported that in many soils applied K fertilizers transformed into non-exchangeable form with the passage of time and makes unavailable for K uptake by the crops (Kansal and Sekhon, 1976).

Effect of INM on yield and proximate composition of colocasia

The highest mean cormel yield of 24.27 t ha^{-1} was recorded with integrated application of FYM along with half of the soil test based NPK with a yield response of 90.7% over the control, followed by $N_{80}P_{30}K_{80}$ (23.57 t ha^{-1}). Incorporation of FYM alone has recorded almost equal yield response in comparison to 80 kg ha^{-1} of fertilizer N or K fertilizers, emphasizing that the application of organic manure alone minimizes the input cost and FYM has beneficial effect rather than chemical fertilizers (Laxminarayana, 2022). Organic manures typically release both macro and micronutrients gradually and supply the crops throughout their growth period and contributed in crop yields (Adediran et al., 2005). Though, the organic sources contain relatively low concentration of nutrients, there has been large increase in their use over inorganic chemical fertilizers for sustaining the productivity of colocasia. The organics help to enhance the retention and availability of all the essential nutrients as well as improve the soil physical and biological properties (Kamara and Lahai, 1997).

The mean cormel yield significantly increased by the application of graded doses of N up to 80 kg N ha^{-1} with yield responses of 35, 57 and 56% over the control due to the addition of 40, 80 and 120 kg N ha^{-1}, respectively. Since the experimental soil contained high status of available P, application of lower doses of P application was advocated. Graded doses of K fertilizers showed an increase in the yield response up to 120 kg K_2O ha^{-1}. Relatively higher yield response was observed with the application of K fertilizers rather than N as the crop responded to higher doses of K fertilization in the experimental sandy loam soil, which contained medium status of available K. Dual application of $N_{80}P_{30}$, $N_{80}K_{80}$ and $P_{30}K_{80}$ showed an increase of 51, 74 and 60% yield respectively over the control. Colocasia yields increased considerably due to the application of higher doses of NPK fertilizers in low and marginally fertile soils as it showed significant response to optimum doses of NPK rather than single application of higher doses of N, P and K. These results are in accordance with the findings of Halavatau et al., (1998).

Effect of organic and inorganic nutrients on proximate composition of colocasia

In the cormels of colocasia, significantly higher mean dry matter (24.67%), starch (12.92%) and total sugars (1.26%) were recorded for the treatment which involved soil test based application of 80-30-80 kg ha^{-1} of N, P_2O_5 and K_2O at par with FYM + ½ NPK (Table 2). Graded doses of N and K fertilizers up to 120 kg ha^{-1} showed an increase in the biochemical constituents. The highest mean starch content was recorded for the application of $N_{80}P_{30}K_{80}$ (12.92%) followed by FYM + $N_{40}P_{15}K_{40}$ (12.87%). Starch content in the cormels ranged from 10.86 to 12.92% and total sugars from 0.86 to 1.26%, with the highest being recorded for the integrated application of FYM + $N_{40}P_{15}K_{40.}$ Incorporation of organic manure enhanced the starch content, which might be due to the increased rate of mineralization of the organic manure resulting in nutrient transformations and their mobility into the plant system (Nizamuddin et al., 2003).

Relationship between yield and biochemical constituents with inorganic fractions of NPK

Among the inorganic N fractions, NH_4-N showed more significant relationship with the cormel yield as well as the biochemical constituents of colocasia ($r=0.892^{**}$) than NO_3-N ($r=0.845^{**}$). Of all the inorganic P fractions, Fe-P had highly significant relationship with cormel yield ($r = 0.806^{**}$), whereas Al-P showed highly significant relationship with starch, sugars and dry matter ($r = 0.804^{**}$, 0.855^{**} and 0.878^{**}, respectively), indicating that both the Fe-P and Al-P greatly contributed the yield and proximate composition of the crop.

Significantly positive correlation was observed between total K and corm yield, starch, total sugars and dry matter

Table 4. Correlation coefficients (r) between cormel yield and biochemical constituents of colocasia with inorganic fractions of nitrogen, phosphorus and potassium

Inorganic nutrient fractions	Mean cormel yield	Starch	Total sugars	Dry matter
Nitrogen fractions				
Total N	0.902**	0.862**	0.909**	0.843**
Available N	0.892**	0.923**	0.937**	0.909**
Ammoniacal N	0.892**	0.845**	0.872**	0.854**
Nitrate N	0.845**	0.831**	0.832**	0.792**
Phosphorus fractions				
Total P	0.675**	0.622*	0.643*	0.705**
Available P	0.781**	0.788**	0.828**	0.854**
Water Soluble P	0.781**	0.788**	0.828**	0.854**
Fe-P	0.806**	0.779**	0.848**	0.845**
Al-P	0.789**	0.804**	0.855**	0.878**
Ca-P	0.600*	0.650**	0.746**	0.701**
RS-P	0.704**	0.723**	0.799**	0.804**
Potassium fractions				
Total K	0.929**	0.963**	0.918**	0.962**
Available K	0.921**	0.944**	0.915**	0.942**
Water soluble K	0.874**	0.938**	0.882**	0.938**
Exchangeable K	0.911**	0.924**	0.903**	0.922**
Non-Exchangeable K	0.943**	0.939**	0.917**	0.948**

* $P < 0.05$; ** $P < 0.01$

and the 'r' values were found to be 0.929**, 0.963**, 0.918**, 0.962**, respectively. The correlation between total K and yield as well as biochemical constituents was more significant than that between total N or total P and yield/ biochemical constituents indicating that enhancement in total K and its fractions in the soil mostly contributed towards the yield and quality of tuber crops in general and colocasia in particular. Addition of potassium fertilizers based on soil test values and crop requirement are very essential to boost the productivity of tuber crops. Of all the K fractions, non-exchangeable or fixed K recorded higher 'r' values with cormel yield (r=0.943**), sugars (r=0.917**) and dry matter (r=0.948**), indicating that non exch. K transformed into exchangeable and water soluble K fractions for maintaining the equilibrium between different fractions and contributed towards the enhancement of yield and quality parameters of the crop. Water soluble K which is the readily permeable form of K nutrition of the crop also showed significant relationship with the yield and quality of colocasia rather than exchangeable K. These results are in accordance with the findings of Srinivasa Rao et al., (2014).

Conclusions

Application of half of the recommended doses of NPK fertilizers in combination with organic manure (FYM) enhanced the efficiency of applied chemical fertilizers that resulted in sustainable productivity and proximate composition of colocasia as well as the nutrient transformations in acid Alfisols of Eastern India. Application of soil test based N, P and K fertilizers was found equally effective in obtaining higher crop yields, nutrient use efficiency, and residual soil fertility. The tuber crops showed greater response to graded doses of K application rather than N fertilization. Different inorganic fractions of nutrients significantly influenced the cormel yield, biochemical constituents of cormels and the residual fertility status of the soil, indicating that the inorganic fractions as influenced by organic sources combined with inorganic fertilization in the present context of climatic scenario helps to enhance the productivity of tropical tuber crops and to maintain the soil quality.

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Molecular identification of *Araecerus fasciculatus* and its endosymbiotic bacteria

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Abstract

Araecerus fasciculatus are one of the important storage pests of cassava, associated with a wide variety of bacterial endosymbionts that confer many ecologically relevant traits to the host insect. Endosymbiotic bacteria (ESB) play a vital role even in the physiology of the host, hence identification of ESB associated with the storage pests will help to develop important strategies for the management of the noxious pest. In the present study molecular characterization of the *Araecerus fasciculatus* and endosymbiotic bacteria associated with them, was done. By molecular characterization they were identified as *Araecerus fasciculatus* and sequences were deposited at NCBI with accession no OR415335. Further, the genomic DNA was isolated from each of the EPB isolates and PCR amplification of 16S rRNA gene was carried out using universal primers. The 16S rDNA gene sequences of endosymbiotic bacterial isolates were generated by sequencing the PCR product and were aligned with each other by using BioEDIT software. The nucleotide sequences were compared with those in the NCBI databases using the Basic Local Alignment Search and were identified as were confirmed as *Bacillus megaterium.* The taxonomy had been recently changed and was renamed *Priestia megaterium*, which is a new separate genus from *Bacillus*. The 16S rRNA gene sequences were also deposited at NCBI database with accession no OR418413. From the aligned sequences phylogenetic tree was constructed by the Neighbor-Joining method using MEGA version 11.

Keywords: *Araecerus* sp., Endosymbiotic bacteria, 16S rRNA, Pests, Symbiosis

Introduction

Endosymbiotic bacteria and fungi associated with the insect host play important role in digestion of food, protection against pathogens, parasites, predators, modulating the interaction of phytophagous insects with host plants and in inter and intraspecific communication (Alvarado et al., 2021). These endosymbionts of insects are classified into primary and secondary endosymbionts. The primary endosymbionts have an obligatory relationship with the insect host, providing essential amino acids and showing phylogenetic congruence with their host. Primary endosymbionts are morphologically similar to each other and are restricted to bacteriocytes (Baumann et al., 2005). The secondary endosymbionts play many functional roles on their hosts such as providing fitness benefits, increasing tolerance to heat stress, increasing resistance to parasitic wasps, causing host plant specialization, conferring invasiveness (Fadden, 2001). The knowledge about interaction between insects and their gut-associated symbionts has importance in agriculture due to their potential application in pest management strategies. Moreover, the insect gut symbionts were capable of enhancing insecticide resistance in several insect species (Kikuchi

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et al., 2012; Xia et al., 2018). Hence, the identification of these endosymbiotic bacteria is gaining more importance for the monitoring and management of chemical insecticide resistance (Cheng et al., 2017). With the increasing number of agriculturally relevant pests, recent studies focus on microbiome-insect mutualism with the help of culture-independent techniques such as shot gun metagenomic studies (Bharti and Grimm, 2019; Gurung et al., 2019). The coffee bean weevil (CBW), *Araecerus fasciculatus* De Geer, (Coleoptera: Anthribidae) is an important pest of stored products such as grains, coffee beans, cassava, and traditional Chinese medicine materials (Yang et al., 2017). A yield loss of up to 20% was caused by the pest in stored and processed cassava products as reported by Chijindu and Boateng, (2008). The weevils were associated with an ancient γ-proteobacterial lineage *Nardonella* which are localized to bacteriomes (Lefevre et al., 2004; Conord et al., 2008; Hosokawa and Fukatsu, 2020; Hosokawa et al., 2015; Anbutsu et al., 2017). In some of the *Sitophilus* grain weevils *Nardonella* was replaced by γ-proteobacterial *Sodalis* endosymbionts and in Curculionini *Acorn* weevils was occupied by γ-proteobacterial *Curculioniphilus* endosymbionts (Heddi et al., 1999; Heddi and Nardon, 2005; Vigneron et al., 2014, Toju et al., 2010, Toju et al., 2013; Toju and Fukatsu, 2011). Hence the identification of the functional role of these primary as well as secondary endosymbionts from insects is important as they play vital role in the lifecycle of the insects. The exclusion of these bacteria may reduce their lifespan and suppress population within few days or weeks.

Materials and Methods

Collection of insects

Adult insects were collected and maintained at the Insect Microbiology Laboratory, ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India. Earlier studies used morphological keys for organism identification. However, in most of the recent studies, identification and confirmation of insects pests are usually done by molecular techniques.

DNA isolation from insect

The genomic DNA was isolated using the modified cetyl trimethyl ammonium bromide buffer (CTAB) method (Gawel and Jarrett,1991). The individual insect samples was homogenized with 500 µl of lysis buffer (CTAB 2%, 100 mM Tris-HCI (pH 8.0), 1.4 M sodium chloride, 20 mM EDTA, 0.1% of 2-mercaptoethanol) and suspended in the same buffer. The suspension was incubated at 65°C for 1 hr and centrifuged at 10,000 rpm for 10 min. Then an equal volume of chloroform: isoamylalcohol (24:1) was added and the suspension was centrifuged at 6000 rpm for 15 min at room temperature. The upper aqueous layer was transferred to a fresh micro centrifuge tube and DNA was precipitated by adding 40 µl of sodium acetate, 600 µl of 95% ethyl alcohol. The tubes were kept at -20ºC for 20 min and centrifuged at 8000 rpm for 10 min. The supernatant was discarded, and the resultant pellet was washed with 70% ethanol, dissolved in 50 µL DNase-, RNase- and Protease-free molecular biology water. The intact genomic DNA was further quantified using Nanodrop ND-1000 (Thermo Scientific, Belgium). The DNA samples were diluted with sterile water to get a working solution of 50-100 ng/µL.

Polymerase Chain Reaction and DNA sequencing

The polymerase chain reaction (PCR) was carried out in a thermal cycler (BioRad, Veriti 96 wells) with the following cycles: initial denaturation 94°C for 5 min as followed by 35 cycles of denaturation 94°C for 45 sec, annealing 47°C for 45 sec, extension 72°C for 45 sec and final extension 72°C for 10min, hold at 4°C. The primers used were specific to mitochondrial cytochrome oxidase (COX-1) F- LCO (GGT CAA CAA ATC ATA AAG ATA TTG G), R- HCO (TAA ACT TCA GGG TGA CCAAAA AAT CA). The PCR analysis was performed in 25 µL total reaction volume containing 20 Pico moles of each primer, 1.0 µL of 20 mM dNTP, 2.5 µL of 10X buffer and 1.0 µL of 1.0 U Taq DNA polymerase (Fermentas Life Sciences, Maryland, USA). The amplified products were resolved in 1.0% agarose gel, stained with ethidium bromide (10 µg ml^{-1}) and visualized in a gel documentation system (UVP). The PCR amplified fragments were eluted using Nucleospin® Extract II (Thermo Scientific, USA). The purified PCR products were sent for sequencing. Sequencing was carried out in an automated sequencer both in forward and reverse directions at Eurofins Genomics India Pvt. Ltd., Bengaluru, Karnataka. Homology search was carried out using BLAST (http://www.ncbi.nlm.nih.gov). From the aligned sequences phylogenetic tree was constructed by the Neighbor - Joining method using MEGA 11 software (Tamura, 2021).

Isolation of ESB

Adult storage pests were collected from the stock culture maintained at the Insect Microbiology Laboratory, ICAR-CTCRI, Thiruvananthapuram were surface sterilized with absolute ethanol. These were homogenized in sterile 0.9% saline and plated directly on to the nutrient agar media and kept for incubation at 30°C overnight under aerobic condition.

Identification of ESB

The pure culture of each ESB was obtained by streaking the individual colony on a fresh nutrient agar plate and incubated for 24 h at 30°C. The colony characters were observed from each separated colony.

Phenotypic characterization of ESB

Cultural characteristics of each bacterium, which include shape, margin and elevation of the isolates of each colony type were observed using stereomicroscope (Carl Zeiss, Stemi 2000C) under 40× magnification, by using research microscope (Leica DMLB) under 100X magnification. Gram staining was done using the Hi- Media kit (Hi-Media Laboratories Pvt. Ltd., India) according to the manufacture's protocol for the identification of unknown bacterial strains collected from the nutrient broth of 24 h culture and were observed under a compound microscope (Leica DMLB) with 100× magnification.

PCR amplification of 16S rDNA of ESB

The PCR amplification of 16S rDNA gene by universal primers: forward primer fD1 5'AGAGTTTGATCCTG GCTCAG3' and reverse primer RP2 5'CGGCTACCTTGTTACGA CTT3' (Weisburg et al., 1991) were used. The PCR was performed in a 25 μl reaction mixture having 2.5 μl of 10X Taq buffer A (containing 15 mM $MgCl_2$, mM each), 1.0 μl of each primer (20 ng), 2 μl of template DNA and 0.25 μl of (1U) Taq DNA polymerase and 17.75 μl of sterile distilled water. The reaction was carried out in a Biorad thermal cycler with the thermal cycle programme of 92°C for 2min 10 s (initial denaturation), 30 cycles at 94°C for 1min 10 s (denaturation), at 49°C for 30 s (annealing), at 72°C for 2 min (extension) and final extension at 72°C for 10 min. The amplified products were resolved on a 1.2% agarose gel. DNA ladder of 500 bp (Bangalore GeNei, India) was used for determining the size of the amplicon. The DNA bands were visualized under UV transilluminator and the purified PCR products of 1500 bp were sequence d at Eurofins Genomics India Pvt. Ltd., Bengaluru, Karnataka, India.

Phylogenetic analysis

The sequences obtained for the EPB isolates were aligned with each other by using Clustal alignment programme of MEGA 11 software (Tamura et al., 2021). The nucleotide sequences were compared with those in the NCBI databases using the Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov/BLAST). From the aligned sequences phylogenetic tree was constructed by the Neighbor - Joining method using MEGA 11 software.

Results and Discussion

Molecular identification of storage pest

Genomic DNA was extracted from the insect samples and the amplification of COX-1 gene was done and product size of amplicon was 658bp. The PCR amplified products were purified and sequenced. The sequences obtained in automated DNA sequencing were aligned and compared by using BioEdit. BLAST analysis of isolates showed 100% similarity to *Chiridopsis* sp. available in the Genbank. The sequence data generated were deposited in the Genbank nucleotide database (NCBI) and the accession numbers assigned are given in Table 1. The phylogentic tree of the isolate based on Mt (COX1) gene sequences is shown in (Fig. 1).

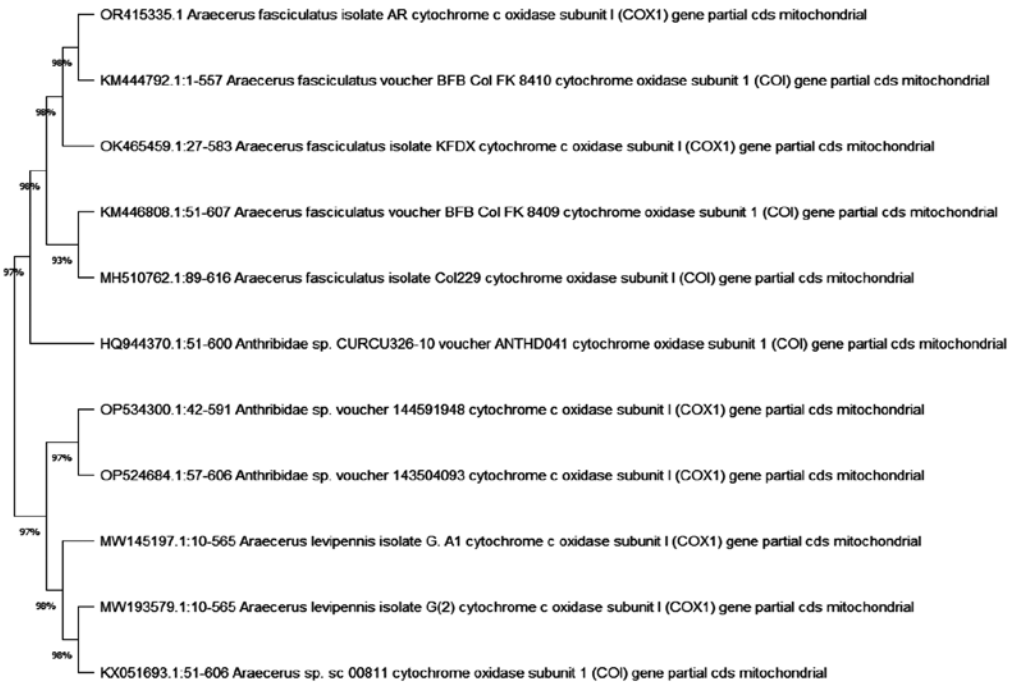

Fig. 1. Phylogenetic tree inferred from mitochondrial cytochrome oxidase (COX-1) sequences analysis of Araecerus fasciculatus isolate AR

Phenotypic and molecular characterization of bacterial isolates

Bacterial symbionts were isolated from the *Chiridopsis* sp. and were assigned code number as isolate A1. Morphological variations were observed for each endosymbiotic bacterial strains, but no pigmentation was observed. Colonies formed on nutrient agar were circular, raised, convex, flat, entire white in colour, gram positive, rod-shaped with no pigmentation. Genomic DNA was extracted from bacterial samples. The PCR amplification of the 16S rDNA of the with the primers 16SF and 16SR at an annealing temperature of 49°C yielded a fragment of approximately 1500 bp. The PCR amplified products were sequenced. BLAST analysis of the sequences of the isolate showed 98% similarity to *Bacillus megaterium* available in the Genbank. The sequence data generated were deposited in the Genbank nucleotide database (NCBI) and the accession numbers assigned are given in Table 1. The phylogentic tree of the endosymbiotic bacteria based on 16S rRNA gene sequences is shown in (Fig. 2).

The taxonomy of *Bacillus* had been recently changed and *Bacillus megaterium* was renamed as *Priestia megaterium*, which is a new separate genus from *Bacillus* (Gupta et al., 2020). Earlier studies have reported *P. megaterium* as a potential biological control agent, with antimicrobial activities and against plant pathogens (Alvarez et al., 2012; Yang, 2019; Nair and Radhakrishnan, 2021). It is also reported that P. *megaterium* can be isolated from various plants, such as alfalfa, black pepper, carrot, clover, cotton, cucumber, potato, wheat, ginseng, dendrobium, and *polygonatum sibiricum* (Afzal et al., 2019; Kłosowski

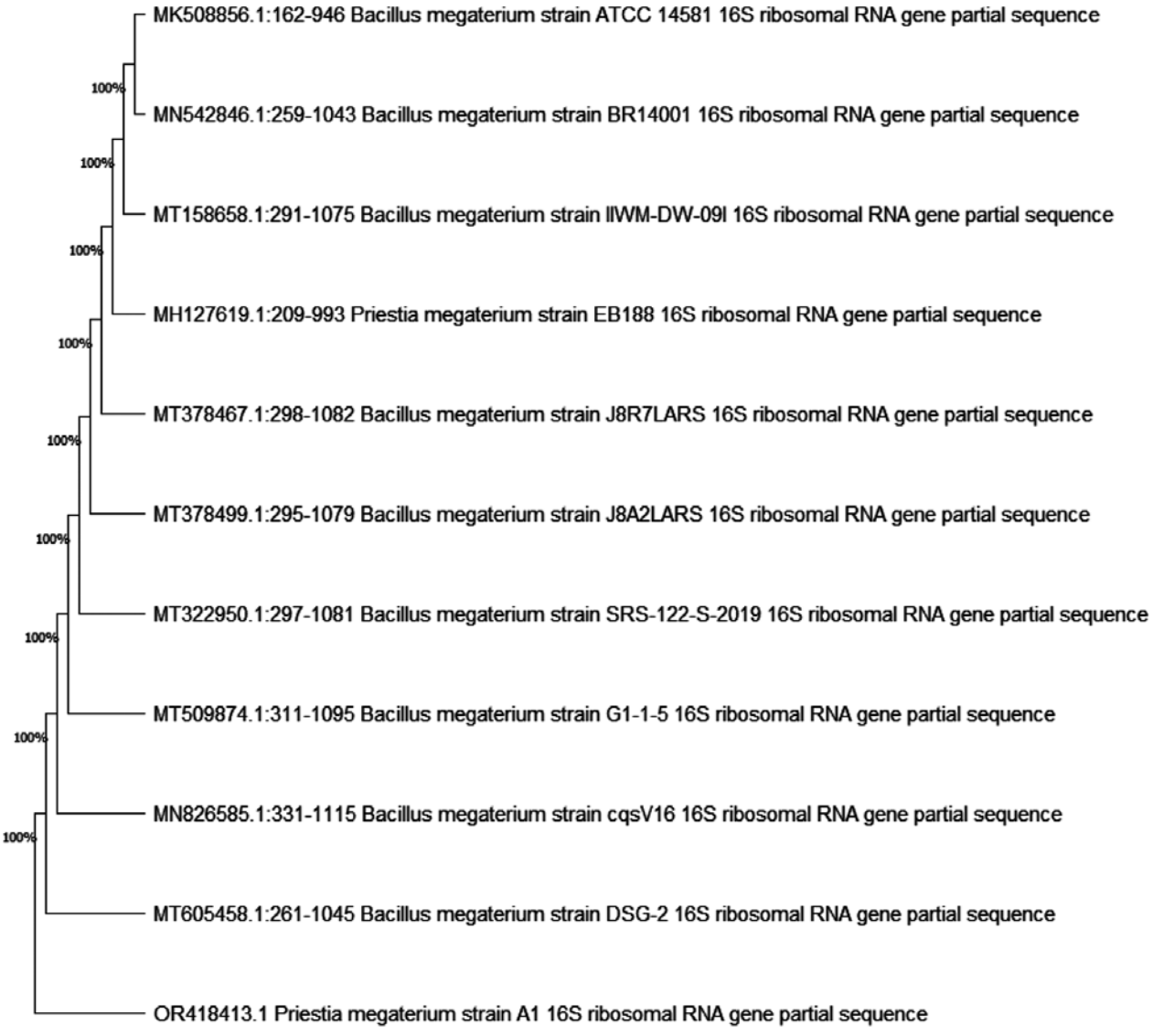

Fig. 2. Phylogenetic tree inferred from 16S rRNA gene sequences analysis of bacterial isolate A1 associated with Araecerus fasciculatus

et al., 2021; Rajan et al., 2021). In our study also, *P. megaterium* was found to be the endosymbiotic bacterium which was associated with the *A. fasiculatus.*

Table 1. Molecular identification of storage pest and endosymbiotic bacteria

Isolate	Identification	Accession No	Similarity (%)
A1	16S ribosomal RNA gene sequence, partial *Priestia megaterium* isolate A1	OR418413	100
AR	Mitochondrial cytochrome c oxidase subunit I (COX1) gene sequence partial *Araecerus fasciculatus* isolate AR	OR415335	98

Salama et al., (2004) have reported the different bacterial isolates *viz.*, *Bacillus sphaericus*, *B. megaterium* and *B. laterosporus*, from infected red palm weevil, *Rhynchophorus ferrugineus. B. megaterium* is also marketed as a crude biofertilizer and studies revealed the ability of the bacteria to promote growth and reduction of diseases in different Indian cultivable plants (Chakraborty et al., 2006). It has also been reported that the bacteria have a probiotic effect on many animals (Jones et al., 2006; Otero et al., 2006). Previous studies also showed the presence of *B. megaterium* from larval tissues of *Ostrinia nubilalis* (Hübner) (Secil et al., 2012). Similar studies also showed the association of *B. megaterium* with lac insects *Kerria lacca* (Kerr) (Gulsaz et al., 2017). *B. megaterium* and organisms belonging to the *B. alvei-B. thiaminolyticus* spectrum were one of the most frequent isolates associated with frass from feral honey bee colonies (Gilliam, 1985). Prvious studies also reported the association of *B. megaterium* with different species of sand flies viz *Phlebotomus papatasi, Lutzomyia evansi, Phlebotomus argentipes* (Vivero et al., 2016; Hillesland et al., 2008; Gunathilaka et al., 2019; Dillon et al., 1996; Mukhopadhyay et al., 2012).

Conclusion

The endosymbiotic bacteria can serve as targets for future functional analysis of diverse genes which are responsible for the various functions within each life stage across different developmental stages of insects. The identification of these bacteria will help to detect the pathway of insect-host interactions and thereby it will help to develop novel pest control strategies.

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This publication covers status papers and Extended Summaries of Research papers presented in the National Seminar on *Amorphophallus* at Patna, Bihar held during July 1920, 2008. This publication is the only one of its kind in *Amorphophallus* (Elephant Foot Yam) which covers 14 status papers on *Amorphophallus* of various states of India and 52 Research papers under different areas namely Bio-diversity, Genetic Resources, Varieties and Biotechnology, Production Technology, Nutrient Management, Water use efficiency, Cropping System, Seed tuber multiplication, Pests and disease management, Biochemistry, Nutraceutical/ Pharmaceutical values and Utilisation, Value added Products, Crop Promotion and Transfer of Technology.

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