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Contents

Research Articles

- Impact of fluctuating actinic high light stress on biomass and yield of cassava 3
Sadasiyan Nair Raji, Saravanan Raju and Velumani Ravi
- Enhancing factor productivity of a greater yam + maize intercropping system under drip fertigation 9
M. Nedunchezhiyan, Kalidas Pati, V.B.S. Chauhan and S.K. Jata
- Phenolic content and *in vitro* bioactivities of Chinese Potato (*Plectranthus rotundifolius*) tuber extracts 16
Megha Madhavan, V.R.Vishnu, S. Shanavas and A.N. Jyothi
- Integrated weed management in elephant foot yam cv. Gajendra 24
Upendra Kumar Naik, Padmakshi Thakur and Adikant Pradhan
- Time series modelling of monthly rainfall in southern Kerala 29
R. S. Neethu and Brigit Joseph
- Productivity and profitability of taro (*Colocasia esculenta* (L.) Schott) under drip and furrow irrigation 36
S. Sunitha and J. Suresh Kumar
- Strategies for enhancing post-harvest quality and shelf life of tuber crops: Insights from physiological perspectives 40
Saravanan Raju
- Performance of improved varieties of cassava in two agroecological units of Kerala 53
R. Muthuraj, James George, S. Sunitha and M.N. Sheela
- Genetic variability for different quantitative characters in colocasia (*Colocasia esculenta* var. *antiquorum*.) 57
Padmakshi Thakur, Upendra Kumar Naik, Vikas Ramteke and Omesh Thakur
- Harnessing the diversity of bacterial endophytes isolated from wild and cultivated taro plants against *Phytophthora colocasiae* 61
Shilpa S.U., Jeeva M. L., Veena S. S., Amrutha P. R., Makesh Kumar and Tom Cyriac

Taro (<i>Colocasia esculenta</i> Schott.) based intercropping systems: interspecies interaction effects on growth and yield	69
M. Nedunchezhiyan, K. Pati, V.B.S. Chauhan, K.H. Gowda, R. Arutselvan S.K. Jata and J. Dixit	
Site-specific nutrient management improves soil quality in an ultisol under continuous cassava cultivation	75
R. Shiny and G. Byju	
Molecular identification of tortoise beetle and its endosymbiotic bacteria	83
B. G. Sangeetha, Gadha Dileep, S. Lekshmi, P. Drishya, E. R. Harish	

Short Communications

Optimisation of callus induction in the leaf and stem tissues of the orange flesh sweet potato variety Bhu Sona	89
Ashna Prasad, Senthilkumar K. M., Vivek Hedge, Krishna Radhika N., Koundinya A.V. V., Shirly R. Anil and Sheela M. N.	
A new nutrient rich biofortified greater yam variety: Gujarat Greater Yam-1 (Hemlata)	93
Himani B. Patel, K. D. Desai, C. G. Intwala and H. R. Rathod	



Impact of fluctuating actinic high light stress on biomass and yield of cassava

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Abstract

Cassava (*Manihot esculenta* Crantz) is a staple crop grown in the tropics for food as a major calorie source as well as in industrial use. In natural environment, crops undergo rapidly changing light conditions which affects the photosynthetic efficiency. When exposed to excess photon flux densities, plants undergo non photochemical quenching (NPQ) by which the excess energy is harmlessly dissipated as heat in order to protect the plants from photo-damage. Upon the transition to low or optimal light for photosynthesis, the slow rate of recovery of NPQ can limit effective photosynthetic efficiency which consequently results in low crop productivity. In the present study, the physiological and fluorescence responses of six field grown cassava genotypes to intermittent high red actinic light (IHL) were examined and compared against control plants grown under ambient light conditions. From the results, it was seen that overall average values of plant height and fresh above ground biomass (ABM) was higher under IHL conditions (206.6 ± 26.5 cm and 2.34 ± 0.67 Kg respectively), while high crop biomass (CBM) was observed in control condition (3.11 ± 0.86 Kg). It was found that Sree Suvarna had the maximum CBM under both the control and IHL conditions (4.31 ± 0.32 Kg and 4.11 ± 0.44 Kg respectively). Higher average values of Pn measured was $34.04 \pm 1.6 \mu\text{mol m}^{-2}\text{s}^{-1}$ (Control - Sree Suvarna), NPQ was 2.12 ± 0.36 (IHL - Sree Athulya) and qN was 0.85 ± 0.03 (Control - Sree Pavithra). Significant difference in fluorescence parameters and crop yield were observed between the light conditions and also between the cassava varieties. It was inferred that IHL has obviously affected the NPQ induction/relaxation process which resulted in reduced CBM compared to that under control condition.

Keywords: Cassava, intermittent high light condition, NPQ induction and relaxation, crop biomass

Introduction

Cassava (*Manihot esculenta* Crantz.) popularly known as tapioca is an important staple food and industrial crop to a large population in Asia, Africa and Latin America. Cassava which is one of the main food source for carbohydrate is a drought resistant crop and is grown mainly by resource limited small scale farmers, which demands for the significance of maximum yield under diverse environmental conditions. Photosynthesis is the

basis of existence of life on earth. Light is one of the most prime requirements for photosynthesis and is also one of the most changing environmental factors. Plants need protection from the excess light greater than $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$ which is usually encountered during sunny days. Several methods are adopted by plants to avoid absorption of excessive light by movement of leaves, adjustments in light harvesting antenna sizes etc (Hirth et al., 2013). Alternative electron transport pathways and

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thermal dissipation methods are also followed by plants which help to remove the excessively absorbed light energy thereby protecting the photosynthetic apparatus (Long et al., 1994; Niyogi, 1999; Zhao et al., 2017). Not all the light falling on leaves is used for photosynthesis and a portion of the incident light is emitted back as fluorescence or wasted as heat. Leaves in the field under natural conditions is exposed to fluctuating sunlight – full sunlight to shade and vice versa as the light intensity received by the plants is controlled by the position and angle of the leaves, time of the day, presence of clouds, presence of wind, its speed and direction and presence of upper leaves of same crop or other crops. Under full sunlight, when the light falling on the leaves is more than that can be used for photosynthesis, non photochemical quenching (NPQ) occurs as a mechanism to protect the photosynthetic apparatus from damaging (Muller et al., 2001). NPQ process involves quenching of singlet excited state Chl and happens via enhanced internal conversion to the ground state which is non radiative decay, in which the excess energy is harmlessly dissipated as heat. On exposure to high and low lights, xanthophylls cycle occurs which reduces the CO₂ fixation with minimal heat dissipation (Holt et al., 2004; Kromdijk et al., 2016; Ruban, 2016). In the xanthophyll cycle, under saturating high light intensity ($>1000 \mu \text{mol m}^{-2}\text{s}^{-1}$), violaxanthin is converted rapidly via the intermediate antheraxanthin to zeaxanthin, which eventually leads to an increase in CO₂ fixation and this reaction is reversed under non saturating low light levels (Demmig Adams and Adams, 1992, 1996) where the zeaxanthin is converted to violaxanthin in the presence of zeaxanthin epoxidase (ZE). In higher plants, NPQ is the central process by which the excess energy is harmlessly dissipated as heat in order to protect the plants from photodamage (Muller et al., 2001).

The importance of NPQ for the protection of the photosynthetic apparatus is supported by its ubiquity in the plant kingdom (Niyogi and Truong, 2013). However, it has been reported that NPQ exerts an effect on the rate of PSII photochemistry by increasing the dissipation of excitation energy by non-radiative processes in the pigment matrices of PSII, which consequently results in a decrease in the efficiency of delivery of excitation energy for PSII photochemistry in low light intensities (Genty et al., 1989). In fact, it has been estimated that the slow reversibility of NPQ can limit the daily canopy carbon uptake of crops grown in the field by up to 30% (Zhu et al., 2004). By mutation in tobacco plants, faster NPQ switching rate was obtained which increased the biomass production by ~15% (Kromdijk et al., 2016).

Rapidly changing light conditions in the field affect carbon gain and plant productivity because photosynthetic responses to these light fluctuations are not instantaneous. So it has become necessary to understand how the plants in changing environmental light conditions acclimate to

light in the field (Lawson et al., 2012). As in the lower light condition, than that required for Pn saturation, the lack of light may limit photosynthesis and the extra time delay taken for the recovery of PSII antenna from the quenched to the unquenched state may affect the productive photosynthesis adversely (Kromdijk et al., 2016). Upon the transition from low to light conditions higher than that required for Pn saturation, the process of carbon fixation is not immediately started and a delay period up to several minutes may occur in the photosynthetic induction process, before attaining full rate of photosynthesis (Rabinowitch, 1956). Hence there exists a trade off between the metabolic cost of photodamage and the reduction in quantum yield due to NPQ and any unbalance in this trade off causes reduction in plant productivity up to 32% (Zhu et al., 2004).

The asymmetry between the rate of change of NPQ induction and NPQ relaxation could be worsened by repeated or prolonged exposure to fast changing light conditions. Consequently, the photosynthetic quantum yield of CO₂ fixation is also transiently depressed as the recovery rate of PS II antennae from the quenched to unquenched state also slows down on transition of incident light from high to low intensity (Kromdijk et al., 2016). It is seen that in several C3 and C4 crop species, a 10% to 15% limitation in photosynthesis occurred upon a slow rate of transition from low to high light as the leaves took time to reach steady state conditions (McAusland et al., 2016). Similarly, on transition from low to high light intensity, there is a delay in time taken for photosynthetic induction to achieve maximum photosynthetic efficiency (Chazdon and Pearcy, 1986; Taylor and Long, 2017). To find the cassava genotypes that are yield efficient under fluctuating light conditions, relation between tuber yield and fluorescence parameters, NPQ and Pn parameters of six different cassava genotypes under IHL and control light conditions was evaluated in this experiment. It was found that plant height and Above ground Biomass (ABM) was higher in IHL plants than in control plants, whereas, Crop Biomass (CBM) was higher in control plants. Also, it was seen that plants subjected to IHL showed higher NPQ and qN values and lower Pn values.

Materials and Methods

Plant growth conditions

Six popular cassava genotypes were selected for the study. All the plants were field grown, fertilized and kept well watered under natural conditions. Stem cuttings were planted with a plant spacing of 1m×1m. After four months, one set of the plants was maintained as control plants and another set of the plants was given additional intermittent high red actinic light (IHL) which provided an additional PAR of $900 \mu \text{mol m}^{-2}\text{s}^{-1}$ (Fluorotronix, 200W, full spectrum Led plant grow light). The lights were mounted using a pole at a height of 1ft above the

top of the plants and the height of the light arrangements was adjusted periodically according to the plant height. In IHL, plants were exposed to high light for a period of 15 minutes followed by ambient light for 15 minutes during the day for the crop growth period. All the gas exchange and fluorescence measurements were taken on fifth fully grown leaf from the top.

Physiological measurements

Licor-6400 portable photosynthesis system (Li-COR Inc, NE, USA) with leaf chamber fluorometer was used for Chlorophyll fluorescence and NPQ measurements. For dark measurements, the leaves were dark adapted by covering using black paper with clip for 20 minutes. This was conducted on plants grown under control (ambient light) condition and IHL condition. The maximum fluorescence (F_m), variable fluorescence (F_v) and the minimal fluorescence (F_o) were measured on the dark adapted leaves. In both control and IHL plants, steady state fluorescence (F_s), maximum fluorescence (F_m') and Variable Fluorescence (F_v') were measured under a fixed external PAR of $3000 \mu\text{mol m}^{-2}\text{s}^{-1}$. All the measurements were done each day between 11.00 and 13.30 hours at ambient day tropical temperature ($30 \pm 2^\circ\text{C}$) at a CO_2 concentration of $350 \text{ mmol mol}^{-1}$ inside the leaf chamber.

Leaf Chlorophyll estimation

Leaf chlorophyll estimation was done using DMSO method. The cut leaves were dissolved in 10 ml of DMSO and kept in oven at 60°C for 1 hour. After 1 hour, the solution was made to cool to room temperature and leaf chlorophyll content was estimated using Thermo Scientific Evolution 201 UV-Visible Spectrophotometer. For the determination of Chl a and Chl b, the following equation was used.

$$\text{Chl a (mg/g FW)} = (14.85 A_{665} - 5.14 A_{648})$$

$$\text{Chl b (mg/g FW)} = (25.48 A_{648} - 7.36 A_{665})$$

Plant height and Biomass

All the plants under control and IHL conditions were harvested and plant height and above ground biomass and crop yield was determined.

Data analysis

Box plots were used to compare the values at control and IHL conditions. The results were subjected to Two way Analysis of Variance (ANOVA) and TUKEY test (Assaad et al., 2015).

Results and Discussion

To investigate the difference on plant growth under control and IHL conditions and also on genotype wise variations, plant height, fresh Above ground Biomass (ABM) and fresh Crop Biomass (CBM) were estimated.

Plant height and ABM were higher in IHL plants than in control plants, whereas, CBM was higher in control plants. Higher overall average values of plant height and ABM observed under IHL conditions were $206.6 \pm 26.5 \text{ cm}$ and $2.34 \pm 0.67 \text{ Kg}$ respectively (Table 2). And highest overall average of CBM was in control plants ($3.12 \pm 0.86 \text{ Kg}$). Variety wise, maximum plant height was measured in M4 (IHL) of $256.7 \pm 15.3 \text{ cm}$, maximum ABM in the variety Sree Swarna (IHL) of $3.06 \pm 1.36 \text{ Kg}$ and maximum CBM in the variety Sree Suvarna (control) of $4.31 \pm 0.32 \text{ Kg}$. (Fig. 1).

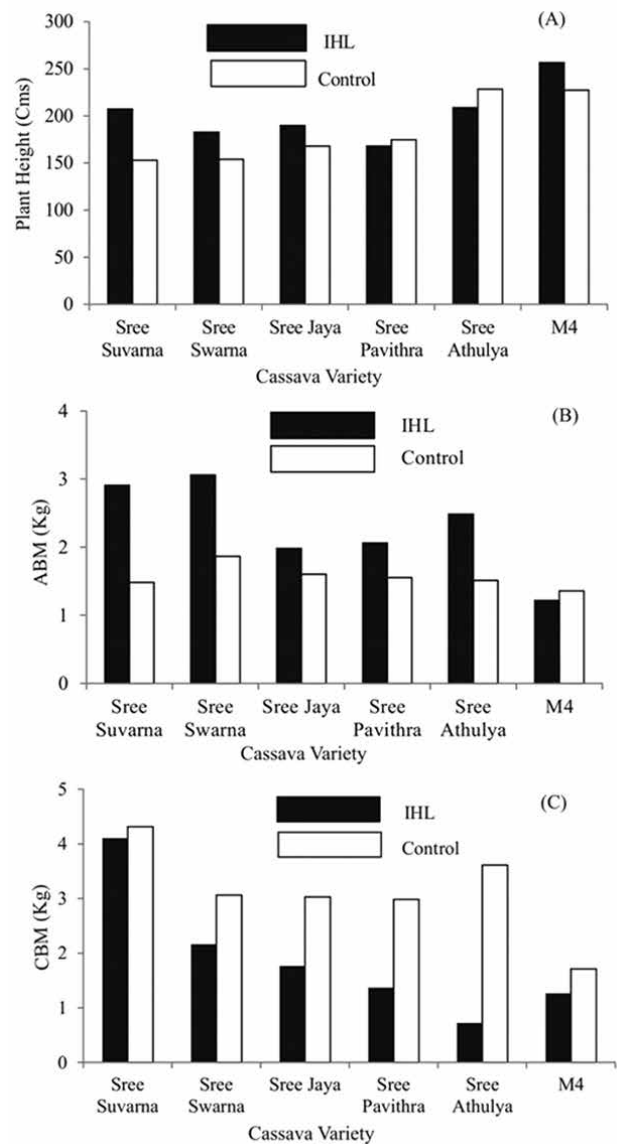


Fig. 1. (A) Plant height, (B) Above ground biomass (ABM), and (C) Crop Biomass (CBM) of cassava varieties under IHL and control conditions. The p -values obtained from 2 Way ANOVA and TUKEY tests were <0.05 both between treatments and between varieties and the interaction of treatment and variety were 0.04, 0.097, 0.57, 0.116 and 0.006.

Table 1. Maximum net photosynthetic rate (Pn), NPQ and qN of cassava leaves grown under control and intermittent high light (IHL) conditions. Values were measured by giving a uniform external PAR of 3000 $\mu\text{mol m}^{-2}\text{s}^{-1}$

Treatment	Variable	Pn ($\mu\text{mol m}^{-2}\text{s}^{-1}$)	NPQ	qN
IHL	Sree Suvarna	31±2.62 ^{ab}	1.49±0.02 ^{ac}	0.78±0.015 ^{ab}
	Sree Swarna	28.2±1.57 ^{ab}	1.67±0.03 ^{ab}	0.84±0.034 ^a
	Sree Jaya	25.3±1.18 ^{ab}	1.75±0.11 ^{ab}	0.81±0.021 ^{ab}
	Sree Pavithra	26.5±3.34 ^{ab}	1.95±0.15 ^{ab}	0.85±0.027 ^a
	Sree Athulya	28.8±0.86 ^{ab}	2.13±0.22 ^a	0.82±0.01 ^{ab}
	M4	22.8±2.95 ^b	1.64±0.1 ^{ac}	0.83±0.014 ^{ab}
Control	Sree Suvarna	34±0.95 ^a	0.99±0.24 ^c	0.71±0.049 ^{bc}
	Sree Swarna	32.4±1.31 ^a	1.57±0.02 ^{ac}	0.63±0.034 ^c
	Sree Jaya	31.2±0.97 ^{ab}	1.76±0.18 ^{ab}	0.79±0.009 ^{ab}
	Sree Pavithra	28.2±1.11 ^{ab}	1.43±0.08 ^{bc}	0.8±0.021 ^{ab}
	Sree Athulya	32.7±1.8 ^a	2.06±0.02 ^{ab}	0.8±0 ^{ab}
	M4	31.1±1.31 ^{ab}	1.67±0.10 ^{ab}	0.80±0.013 ^{ab}
P value	Treatment	<0.001	0.017	<0.001
	Var	0.043	<0.001	0.002
	T×V ¹	0.57	0.116	0.006

Table 2. Plant height, ABM, Crop BM, Leaf Chl a and Leaf Chl b of cassava leaves grown under control and intermittent high light (IHL) conditions.

Treatment	Variable	Plant height	ABM	Crop BM	Chl a	Chl b
IHL	Sree Suvarna	207±6.36 ^{ab}	2.91±0.082	4.11±0.25 ^{ab}	0.93±0.04 ^{de}	0.2±0 ^{ac}
	Sree Swarna	183±6.36 ^{ab}	3.06±0.784	2.16±0.64 ^{ac}	0.48±0.04 ^{fg}	0.07±0.01 ^d
	Sree Jaya	190±31.2 ^{ab}	1.98±0.681	1.77±0.63 ^{ac}	0.83±0.12 ^{def}	0.11±0.02 ^{cd}
	Sree Pavithra	194±30.4 ^{ab}	2.39±0.921	1.88±0.62 ^{ac}	0.87±0.06 ^{def}	0.13±0.01 ^{cd}
	Sree Athulya	209±17.3 ^{ab}	2.48±0.228	0.72±0.27 ^c	0.43±0.02 ^g	0.06±0.0 ^d
	M4	257±8.82 ^a	1.22±0.149	1.27±0.33 ^{bc}	0.76±0.01 ^{eg}	0.12±0.00 ^{cd}
Control	Sree Suvarna	153±19.1 ^b	1.48±0.084	4.31±0.18 ^a	1.61±0.05 ^a	0.29±0.03 ^a
	Sree Swarna	154±5.51 ^{ab}	1.87±0.384	3.06±0.19 ^{ac}	1.43±0.04 ^{ab}	0.18±0.01 ^{bc}
	Sree Jaya	168±15.2 ^{ab}	1.6±0.197	3.03±0.65 ^{ac}	1.4±0.10 ^{ac}	0.18±0.08 ^{bc}
	Sree Pavithra	175±9.6 ^{ab}	1.55±0.055	2.98±0.23 ^{ac}	1.36±0.18 ^{ac}	0.24±0.03 ^{ab}
	Sree Athulya	228±21.6 ^{ab}	1.51±0.066	3.61±1.27 ^{ab}	1.03±0.01 ^{ce}	0.18±0.01 ^{bc}
	M4	227±37 ^{ab}	1.36±0.366	1.71±0.45 ^{ac}	1.19±0.06 ^{bcd}	0.14±0.02 ^{cd}
P value	Treatment	0.068	0.006	0.002	<0.001	<0.001
	Var	0.009	0.193	0.002	<0.001	<0.001
	T×V ¹	0.625	0.535	0.252	0.04	0.097

Values are means ± SEM, n = 3 per treatment group. ^{a-g}Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test. Different superscript letters indicate significant differences ($P \leq 0.05$) between Control and IHL treatments $T \times V^1 = \text{Treatment} \times \text{Variety}$ interaction effect.

Higher growth (plant height and above ground biomass) observed in IHL plants are mainly because of the higher daily dose of irradiant light in IHL plants compared with the control plants (Wagner et al., 2006). Lower crop productivity obtained under IHL is attributed to the

close correlation between the rate of recovery from the photoprotected state and the biomass production when the plants are subjected to periodical light fluctuation (Wang et al., 2002). A lagging response of photosynthesis occurs on occurrence of light fluctuation, which may

consequently result in limitation of crop productivity (Slattery et al., 2018). Any change in light intensity, even if it is for a few seconds may cause change in plant photosynthesis (Yamori et al., 2016).

Plants subjected to IHL showed an increase in NPQ and qN and significantly lower Pn compared to the corresponding values of control plants, when measured at a uniform PAR of $3000 \mu \text{mol m}^{-2} \text{s}^{-1}$. Highest Net photosynthetic rate (Pn) was observed in the variety Sree Suvarna under both control ($34.05 \pm 1.64 \mu \text{mol m}^{-2} \text{s}^{-1}$) and IHL conditions ($30.96 \pm 4.54 \mu \text{mol m}^{-2} \text{s}^{-1}$), with the same variety measuring highest CBM (Control- $4.31 \pm 0.32 \text{ Kg}$). From the results, it can be seen that higher measured Pn explains for the obtained high productivity. Higher NPQ was observed in leaves exposed to IHL indicating that more energy was dissipated as heat, indicating that these leaves suffered photoinhibition (Table 1). Similar to NPQ, qN also showed higher values in IHL plants with the overall average values of NPQ as (1.58 ± 0.36 and 1.76 ± 0.23 in control and IHL respectively) and qN as (0.76 ± 0.07 and 0.82 ± 0.03 in control and IHL respectively). Highest crop biomass obtained, correlated with the highest leaf Chl a and Chl b values measured in the variety Sree Suvarna with values Chl a of 1.61 ± 0.09 (control) and 0.93 ± 0.06 (IHL). Correspondingly, maximum Chl b was measured in the same variety with values 0.29 ± 0.06 and 0.20 ± 0.01 under control and IHL respectively. Between control and IHL conditions, Chl a, Chl b, Pn, NPQ, qN, ABM and CBM showed significant difference, whereas, between the varieties except ABM all the parameters showed significant difference. Interactive effect of Treatment over cassava varieties was significant only for Chl a and qN.

Lower values observed in leaf Chl a and Chl b of IHL plants is also attributed to the increased irradiant light. With increase of average light intensity, decrease in the number of light harvesting units occur which results in the decrease of leaf chlorophyll content (Janssen et al., 2001; Friedman and Alberte, 1986). Under IHL condition, the plants were repeatedly switching between high and ambient light at an interval of 15 minutes each, which caused switching of NPQ induction and relaxation process. Even though plants under control light condition experienced light fluctuations upon cloud covering or upper leaf movement, compared to the IHL plants, the variation was much less. In a longer term, the time delay between NPQ induction and relaxation was intensified by repeated exposure to high and ambient light in IHL plants and consequently the time taken for PSII reversibility on high to low light transition resulted in low crop yield (Long et al., 1994; Zhu et al., 2004).

The enhancement in NPQ under IHL condition is apparently associated with dissipation of photon energy by NPQ, thus preventing damage to the photochemical pathway before the energy is accumulated as reactive

intermediate substances in the photosynthetic chain (Li et al., 2014; Ralph et al., 2002). Increase in NPQ is also attributed to the xanthophylls cycle activity (Ruban, 2016). At a PAR of $3000 \mu \text{mol m}^{-2} \text{s}^{-1}$, the plants under IHL were exposed to additional light intensity which further increased the NPQ response and hence higher NPQ values were obtained when compared to those under control light condition.

Conclusion

Non uniformity of environmental conditions and fluctuation in light is inherent in nature. It is seen that the plants have natural mechanisms to improve protection process, when light increases. Even though, higher plant growth and above ground biomass was found higher in IHL plants, higher crop biomass was obtained in plants under ambient light conditions. In IHL plants, variation in light intensity caused either NPQ induction or relaxation which consequently reduced the photosynthetic efficiency. Regardless of application of IHL, the plants were not able to properly utilize the extra light energy in terms of crop yield which consequently reduced the tuber biomass. It can be concluded from this study that plants grown under IHL condition had greater plant growth and above ground biomass, but had low crop productivity. This signifies the relation between NPQ variation with fluctuating light and crop productivity. This study was done on six popular varieties of cassava and the result showed that the variety Sree Suvarna which has higher crop yield at control condition ($4.31 \pm 0.32 \text{ Kg}$), also has higher crop yield under IHL condition ($4.11 \pm 0.44 \text{ Kg}$) and found to be tolerant to light fluctuations. Significant difference in fluorescence parameters and crop yield were observed between the light conditions and also between the cassava varieties. Further detailed research could be done to evaluate more cassava varieties that shows good tolerance to light fluctuation and has better performance in terms of crop yield under varying light condition.

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Enhancing factor productivity of a greater yam+maize intercropping system under drip fertigation

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Abstract

Field experiments were conducted for consecutive two years (2015-16 and 2016-17) at the Regional Station of ICAR-Central Tuber Crops Research Institute, Dumuduma, Bhubaneswar, Odisha to study the effect of drip irrigation and fertigation levels on factor productivity of a greater yam + maize intercropping. Drip irrigation treatments: $I_1=80\%$ cumulative pan evaporation (CPE) during 1-270 days after planting (DAP); $I_2=100\%$ of CPE during 1-90 DAP+80% of CPE during 91-270 DAP and $I_3=100\%$ of CPE during 1-270 DAP were included in main plots. Fertigation treatments: $F_1=100-90-100$ kg ha⁻¹; $F_2=120-90-120$ kg ha⁻¹; $F_3=140-90-140$ kg ha⁻¹ and $F_4=160-90-160$ kg ha⁻¹ of N-P₂O₅-K₂O were included in sub plots. A control (surface flood irrigation at 100% of CPE and soil application of N-P₂O₅-K₂O 120-90-120 kg ha⁻¹) was included to compare drip fertigation treatments. Treatment I_3 resulted in maximum maize yield; I_2 resulted in maximum greater yam and tuber equivalent yield (TEY). Fertigation at F_4 was resulted in higher maize and greater yam yield and TEY than other treatments. Treatments I_2F_4 and $I2F3$ were on par and resulted in higher greater yam yield, TEY, nutrient and water use efficiency. The treatments control and I_1F_2 resulted in same level of TEY, which indicated saving of 0.684-0.710 million litre (17.9-25.9%) of water per ha under drip irrigation. Same level of TEY with the treatments viz., control and I_2F_1/I_3F_1 also indicated a saving of nutrients N-K₂O 20-20 kg ha⁻¹ (20%) under drip irrigation over soil application. The treatment I_2F_3 (drip irrigation at 100% of CPE during 1-90 DAP+80% of CPE during 91-270 DAP along with fertigation of N-P₂O₅-K₂O 140-90-140 kg ha⁻¹) is recommended for greater yam + maize intercropping system considering greater TEY, nutrient and water use efficiency as well as minimum water requirement per kg of TEY production.

Keywords: *Dioscorea alata*, *Zea mays*, Consumptive use, Nutrient use efficiency, Tuber equivalent yield, Water use efficiency

Introduction

Greater yam (*Dioscorea alata* L.) + Maize (*Zea mays* L.) is a popular intercropping system in high rainfall regions of India. Greater yam is a trailing herb and needs staking. In greater yam + maize intercropping system, maize grain cobs are harvested at physiological maturity and haulms

are left in the field to serve as live staking. Water and nutrients are the most important input factors in crop production which constraint productivity of the crops. Availability of water to agriculture is decreasing due to increasing demand in industrial and allied sectors. Hence, water should be used judiciously in crop production along with rainwater. Rainfall in India is monsoon dependent

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and 80% of rainfall is received during southwest monsoon season, June to September. Maize, being a short duration (90-100 days) crop, can be successfully cultivated in high rainfall regions under rainfed conditions. Greater yam, being a long duration (9-10 months) crop, may suffer from insufficient moisture at later stages. Supplementary irrigation to greater yam + maize intercropping system is beneficial for uniform sprouting at early stage and rapid tuber bulking during post monsoon season. The most used method of irrigation is surface flood irrigation which ensures uniform spreading, high evaporation and seepage loss. Greater yam + maize intercropping system develops thick canopy 3 months after planting and causes difficult for application of surface flood irrigation. Further, water is a scarce resource which needs to be preserved. Drip irrigation is an efficient method of providing water directly to the plant root zone. Irrigation efficiency in drip irrigation is as high as 90% compared to 30-50% in surface irrigation with a saving of water of 40-80% (Dhawan, 2002). Greater crop yields with saving of water and higher water use efficiency in vegetables occurs with drip irrigation systems (Manjunath et al., 2001; Tiwari et al., 2003; Montazar et al., 2019). The dense foliage develops in greater yam + maize intercropping system will not interfere in irrigation under drip system.

Nutrients, one of the important input factors in crop production has low use efficiency due to improper time, method and quantity of application. Nutrient management for greater yam + maize intercropping is necessary to achieve high yields. For the greater yam + maize intercropping system, a fertilizer dose of N-P₂O₅-K₂O 100-75-100 kg ha⁻¹ along with mulching (2 t ha⁻¹ dried farm waste) is recommended for economic yield (Nedunchezhiyan et al., 2010). Application of N-P₂O₅-K₂O 120-90-120 kg ha⁻¹ to the greater yam + maize intercropping system resulted in higher greater yam and maize yields (Sahoo et al., 2006). Top dressing of fertilizers in greater yam + maize intercropping is very difficult due to canopy development after the third month. Hence, drip irrigation and fertigation is an option for water and nutrient management for the greater yam + maize intercropping system. Few studies were conducted on nutrient management for greater yam + maize intercropping system in India and elsewhere in the world, but very meager information is available on water and nutrient management through drip irrigation. Keeping in view of the above, an investigation was conducted to evaluate effects of drip irrigation and fertigation on production potential, water and nutrient use efficiency of the greater yam + maize intercropping system.

Materials and Methods

Field experiments were conducted during 2015-2016 and 2016-2017 seasons at the Regional Station

of Indian Council of Agricultural Research ICAR-Central Tuber Crops Research Institute (CTCRI) (20°14'53.25" N, 85°47'25.85" E, 33 m above mean sea level) at Bhubaneswar, Odisha, India. The climate of the experimental site was characterized by a hot, humid summer, and a cool, dry, winter. The soil was an alfisol with 13.6% water content at permanent wilting point, 27.6% water content at field capacity, 1.53 g cc⁻¹ bulk density, 6.8 pH, 0.39% organic carbon, 196 kg ha⁻¹ available N, 21.4 kg ha⁻¹ available P, and 265 kg ha⁻¹ available K in the top 0.30 m. The experiment was laid out in split plot design and replicated 3 times with the drip irrigation treatments: I₁ = at 80% cumulative pan evaporation (CPE) during 1-270 days after planting (DAP); I₂ = at 100% of CPE during 1-90 DAP+80% of CPE during 91-270 DAP and I₃ = at 100% of CPE during 1-270 DAP were in main plots. The fertigation treatments: F₁ = 100-90-100 kg ha⁻¹; F₂ = 120-90-120 kg ha⁻¹; F₃ = 140-90-140 kg ha⁻¹ and F₄ = 160-90-160 kg ha⁻¹ of N-P₂O₅-K₂O were included in sub-plots. The greater yam + maize intercropping system responded up to 90 kg ha⁻¹ phosphorus in the study location as per previous studies (Nedunchezhiyan et al., 2010) and hence held constant in all fertigation treatments. The recommended fertilizer rate for greater yam + maize intercropping system was N-P₂O₅-K₂O 120-90-120 kg ha⁻¹ under soil application (Nedunchezhiyan et al., 2010). Hence, a control treatment (surface flood irrigation at 100% of CPE and soil application of N-P₂O₅-K₂O 120-90-120 kg ha⁻¹) was included to compare the treatments. During the first and second season, greater yam and maize were established on 17 April 2015 and 22 April 2016, respectively. Cut tubers, 200 g of greater yam, var. Da 293, were planted on ridge tops at 5-7 cm depth and 90 cm between plants. In intra-rows, between 2 greater yam plants 3 hybrid maize 'MRM 3777' seed were sown at 2-3 cm depth at 30 cm spacing on the same day. Plant populations of 12345 and 37037 plants ha⁻¹ for greater yam and maize, respectively, were established.

Water soluble N, P and K fertilizers (urea, urea phosphate and potassium sulphate) were split into 5 equal applications (basal, 30, 60, 90 and 120 DAP) and supplied through drip irrigation with emitter spacing of 30 cm and flow rate of 4 L h⁻¹. In control treatment, the full P₂O₅ (single super phosphate) was applied to soil prior to planting. The N (urea) and K (muriate of potash) were applied to soil in 3 split applications, basal (40%), 45 DAP (30%) and 90 DAP (30%). Drip irrigation on alternate days and surface irrigation (treatment) once every 7 days were given as per treatment based on CPE based on pan factor 0.7. Weeding followed by earthing up was done at 30 and 60 DAP. Maize cobs were harvested at physiological maturity (90 DAP) and stalks and leaves left in the field to serve as staking for the greater yam. Maize cobs were harvested on 15 July 2015 and 20 July 2016,

respectively. Irrigation was withheld for 20 days before harvesting of greater yam in all treatments. Greater yam was harvested 290 DAP. During first and second season, greater yam was harvested on 31 January 2016 and 5 February 2017, respectively.

Rainfall received during first (2015-2016) and second (2016-2017) cropping seasons was 980.0 and 1238.5 mm, respectively. During the first cropping season, effective rainfall (Reddy and Reddi, 2010) was 439, 396 and 396 mm at I₁, I₂ and I₃, respectively. The amount of water applied through drip irrigation was 383, 432 and 451 mm at I₁, I₂ and I₃, respectively. During the second cropping season, effective rainfall was 470, 448 and 441 mm at I₁, I₂ and I₃, respectively. The amount of water applied through drip irrigation was 274, 301 and 345 mm at I₁, I₂ and I₃, respectively. In control treatment, 451 and 345 mm of water was applied surface irrigation during the first and second cropping season, respectively. Soil profile moisture contribution was calculated by gravimetric method from one metre soil depth by available soil moisture at the start of the experiment minus available soil moisture at the end of the experiment. The tuber equivalent yield (TEY), consumptive use (CU), water use efficiency (WUE), water required per kg of TEY production (litre) and nutrient use efficiency (NUE) were calculated by following standard methods.

The data collected were subjected to analysis of variance (ANOVA) in split plot as well as randomized block design using statistical software SAS (SAS, 2010). Treatment means were compared for significance at the 0.05 level of probability using the critical differences (CD) as suggested by (Gomez and Gomez, 1984).

Results and Discussion

Maize yield

Drip irrigation and fertigation levels significantly influenced maize yield (Table 1). The treatment I₃ resulted in maximum maize yield, but it was statistically at par with I₂ (Table 1). The increase in maize yield of I₃ over I₂ was negligible, because in both the treatments equal quantity of water was applied during 1-90 DAP (until the harvest of maize at physiological maturity). Better performance of maize in terms of yield in treatments I₂ and I₃ might be presumably due to less competition for water between the greater yam and maize, effective absorption and utilization of available nutrients, and better proliferation of roots with the favourable soil moisture. Increasing fertigation level increased the maize yield (Table 1). Fertigation at F₄ level resulted in significantly higher maize yield than the other treatments. Adequate supply of NPK might have increased chlorophyll formation, cell elongation and division, enzymes involved in various metabolic processes, nucleotide, protein etc. that led to more production and translocation of photosynthates towards sink (Manickasundaram et al., 2002). Drip fertigation levels revealed that maximum maize yield was recorded in I₃F₄ (Table 2). The treatments I₃F₄, I₃F₃, I₂F₄ and I₂F₃ resulted in greater maize yield of 18.5-30.4, 14.8-30.4, 14.8-30.4 and 14.8-26.1% over the control, respectively. Drip fertigation provided water and nutrients directly to the root zone of plants with apparent greater efficiency than surface irrigation with soil application of nutrients. Under surface flood irrigation weeds can be major competitors for water and nutrients. In water and nutrient stressed fields, weeds can absorb water and

Table 1. Effect of drip irrigation and fertigation on yield, CU, WUE and water required per kg of TEY of greater yam + maize intercropping system

Treatment	Maize yield (t ha ⁻¹)		Greater yam yield (t ha ⁻¹)		TEY (t ha ⁻¹)		CU of water (mm)		WUE (kg TEY ha-mm ⁻¹)		Water required per kg of TEY (litre)	
	2015-2016	2016-2017	2015-2016	2016-2017	2015-2016	2016-2017	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17
Drip irrigation												
I ₁	2.6	2.4	31.0	28.9	33.7	31.3	886	805	38.0	38.9	267	261
I ₂	2.9	2.8	34.9	33.8	37.9	36.5	896	815	42.2	44.8	239	227
I ₃	3.0	2.7	33.4	30.6	36.3	33.3	897	833	40.5	40.1	250	252
SEm±	0.05	0.04	0.44	0.36	0.46	0.37	0.9	0.7	0.05	0.43	3.2	2.7
CD (P=0.05)	0.2	0.2	1.7	1.4	1.8	1.5	3	3	2.0	1.7	13	11
Fertigation												
F ₁	2.5	2.3	28.0	26.3	30.5	28.6	891	816	34.2	35.1	294	289
F ₂	2.8	2.6	32.8	30.2	35.6	32.8	892	817	39.9	40.2	251	250
F ₃	3.0	2.8	35.5	33.5	38.5	36.2	894	818	43.1	44.4	233	227
F ₄	3.0	2.9	36.1	34.3	39.1	37.2	894	819	43.7	45.4	229	222
SEm±	0.02	0.03	0.51	0.49	0.52	0.50	0.2	0.3	0.59	0.60	4.6	4.4
CD (P=0.05)	0.1	0.1	1.5	1.5	1.5	1.5	1	1	1.7	1.8	14	13

nutrients more efficiently than the crop (Singh et al., 2014; Nedunchezhiyan, 2017).

Greater yam yield

Greater yam yield was significantly influenced by drip irrigation and fertigation levels (Table 1). The treatment I_2 resulted in maximum greater yam yield compared to other drip irrigation levels (Table 1). The greater yam yield decreased in the treatment I_3 at 4.3-9.6% compared to the treatment I_2 . With excessive soil moisture conditions, plants may develop more vegetative growth by diverting photosynthates towards growing points. Increasing fertigation level increased the greater yam yields (Table 1). Fertigation at F_4 level resulted in maximum greater yam yield. The fertigation treatment F_4 was greater by 1.7-2.4, 10.1-13.6 and 28.9-30.4% than the treatments F_3 , F_2 and F_1 , respectively. Remya and Byju (2020) reported maximum greater yam yield at greater level of nutrient application. The treatment I_2F_4 resulted in superior greater yam yield (Table 2). The effect of drip irrigation and fertigation revealed that at favourable soil moisture without wide fluctuations along with sufficient nutrients available for absorption and utilization resulted in maximum greater yam yield.

Tuber equivalent yield (TEY)

Marked variation in TEY was noticed with respect to drip irrigation and fertigation levels (Table 1). Increasing drip irrigation increased TEY up to I_2 level which afterwards decreased. This was due to both maximum

maize and greater yam yield in I_2 treatment (Table 1). The drip irrigation at I_2 has coincided with the water requirement of greater yam and maize. In this treatment drip irrigation at 100% of CPE during 1-90 days could meet the water requirement of maize and greater yam. Subsequently drip irrigation at 80% of CPE during 90-270 days was sufficient for greater yam growth and yield. Thus, the treatment I_2 resulted in greater TEY than other treatments. Fertigation at F_4 level resulted in maximum TEY whereas the minimum TEY was noticed in plants under the treatment F_1 (Table 1). This was due to more maize and greater yam yield (Table 1). The treatment F_4 resulted in 1.6-2.5, 10.0-13.1 and 28.2-30.1% greater TEY than the treatments F_3 , F_2 and F_1 , respectively. Decreased yield response to successive increase of nutrient levels have been reported in many crops (Behera and Ghosh, 2009). The drip fertigation treatments I_2F_4 , I_2F_3 , I_3F_4 and I_3F_3 and I_2F_2 resulted in greater TEY (Table 2) due to adequate soil moisture during the crop growing period which increased the availability of applied nutrients to the greater yam and maize. Although in the control treatment the quantity of water applied through surface irrigation was equal to I_3 and nutrients applied in soil was equal to F_2 , the TEY was lower than I_3F_2 . This was because of loss of water and nutrients apart from heavy weed infestation which removed considerable amount of water and nutrients from the soil. The TEY of control treatment was statistically at par with I_1F_2 , I_2F_1 and I_3F_1 . This indicated that surface irrigation with soil application of fertilizer (control) and drip irrigation at I_1

Table 2. Drip fertigation influence on yield, CU, WUE and water required per kg of TEY of greater yam + maize intercropping system

Treatment	Maize yield (t ha ⁻¹)		Greater yam yield (t ha ⁻¹)		TEY (t ha ⁻¹)		CU of water (mm)		WUE (kg TEY ha ⁻¹ mm ⁻¹)		Water required per kg of TEY (litre)	
	2015-2016	2016-2017	2015-2016	2016-2017	2015-2016	2016-2017	2015-16	2016-17	2015-16	2016-17	2015-16	2016-17
I_1F_1	2.3	2.1	25.4	24.1	27.6	26.2	885	804	31.2	32.6	292	308
I_1F_2	2.6	2.5	30.8	28.9	33.4	31.3	886	805	37.8	39.0	252	257
I_1F_3	2.8	2.4	33.8	31.0	36.6	33.4	887	806	41.2	41.7	230	240
I_1F_4	2.8	2.7	34.2	31.5	37.0	34.1	887	806	41.7	42.3	225	236
I_2F_1	2.6	2.4	30.6	28.5	33.2	30.9	895	814	37.0	38.0	270	268
I_2F_2	2.9	2.7	34.9	32.0	37.8	34.7	895	814	42.2	42.7	237	235
I_2F_3	3.1	2.9	36.9	37.0	40.0	39.9	896	815	44.6	49.0	224	205
I_2F_4	3.1	3.0	37.3	37.5	40.4	40.6	897	816	45.0	49.7	223	202
I_3F_1	2.6	2.4	28.2	26.3	30.8	28.7	895	831	34.4	34.7	321	290
I_3F_2	2.9	2.7	32.8	29.7	35.5	32.4	896	832	39.8	39.0	265	257
I_3F_3	3.1	2.9	35.8	32.4	38.9	35.4	898	834	43.4	42.5	243	236
I_3F_4	3.2	3.0	36.7	33.9	39.9	36.9	899	834	44.4	44.2	240	227
Control	2.7	2.4	29.0	27.5	31.8	29.9	899	835	35.3	35.8	283	280
SEm±	0.05	0.06	0.86	0.80	0.88	0.81	0.8	0.7	0.99	0.98	7.4	7.0
CD (P=0.05)	0.2	0.2	2.5	2.3	2.6	2.4	2	2	2.9	2.8	22	21

with fertigation of same level of fertilizer (F_2) resulted in the similar TEY. Thus, drip irrigation I_1F_2 saved 0.684-0.710 million litre (17.9-25.9%) of water per ha. Same level of TEY in the control and in the treatments I_2F_1/I_3F_1 indicated a saving of nutrients N-K₂O 20-20 kg ha⁻¹ (20%). Patil et al. (2011) reported 30% fertilizer saving when fertilizer was applied through drip irrigation in sweet corn.

Consumptive use (CU) of water

The CU of water was greater with the increase in drip irrigation levels (Table 1). The treatment I_1 resulted in lower CU, whereas I_3 resulted in greater CU. The CU during the first season was higher than the second season. This might be due to greater temperature prevailed during the first season which caused greater evaporative demand. This was amply indicated by the quantity of irrigation water applied to the crop. The CU in greater yam + maize intercropping system increased with increase in fertigation levels (Table 1). The treatment F_4 resulted in greater CU compared with the other treatments. The effect of drip fertigation levels indicated greater CU was in the treatment I_3F_4 (Table 2). This might be due to greater quantity of water irrigated and evapo-transpiration.

Water use efficiency (WUE)

The WUE of greater yam + maize intercropping system was significantly influenced by both the drip irrigation and fertigation levels (Table 1). The WUE increased with increase in drip irrigation level up to I_2 and then decreased (Table 1). The WUE decreased at the treatment I_3 by 4.0-10.5% than the treatment I_2 due to lower TEY with the cost of higher shoot biomass. The CU of water and WUE were in quadratic relationship (Fig. 1). Increasing

in CU increased WUE and then decreased. Arora et al. (2007) reported that WUE increased from no irrigation to partial irrigation regime and decreased thereafter with more irrigation. Increasing the fertigation level increased the WUE. The treatment F_4 resulted in significantly higher WUE than F_1 and F_2 , but on par with F_3 . The treatment F_4 resulted in greater WUE of 1.4-2.3, 9.5-12.9 and 27.8-29.3% than the treatments F_3 , F_2 and F_1 , respectively. The effect of drip fertigation levels on WUE of greater yam + maize intercropping system revealed that the treatments I_2F_4 , I_2F_3 , I_3F_4 , I_3F_3 , I_2F_2 , I_1F_4 , I_1F_3 and I_3F_2 resulted in greater WUE of 27.5-38.8, 26.3-36.9, 23.5-25.8, 18.7-22.9, 19.3-19.5, 18.1-18.2, 16.5-16.7 and 12.7% than the control, respectively (Table 2).

The water required to produce a kg of TEY decreased with increasing drip irrigation level up to I_2 and then increased at I_3 (Table 1). The treatment I_2 saved 11-25 and 28-34 litre of water than the treatments I_3 and I_1 , respectively to produce a kg of TEY. This might be due to greater TEY at optimum level of drip irrigation (Table 1). The water required to produce a kg of TEY decreased with increasing fertilizer level (Table 1). The treatment F_4 resulted in minimum water required per kg of TEY and saved 4-5, 22-28 and 65-67 litre of water than the treatments F_3 , F_2 and F_1 , respectively to produce a kg of TEY. This might be due to higher yield of maize and greater yam. The treatments I_2F_4 and I_2F_3 saved 60-78 and 59-75 litre respectively, to produce one kg. of TEY compared to the control (surface irrigation with recommended dose of fertilizer) (Table 2).

Nutrient use efficiency (NUE)

The NUE increased with increasing drip irrigation level up to the treatment I_2 and then decreased at I_3 (Fig. 2). The treatment I_2 resulted in maximum NUE owing to greater TEY. The NUE increased with increasing fertilizer level up to F_2 and then declined (Fig. 3). The treatment F_2 resulted in greater NUE. This might be due

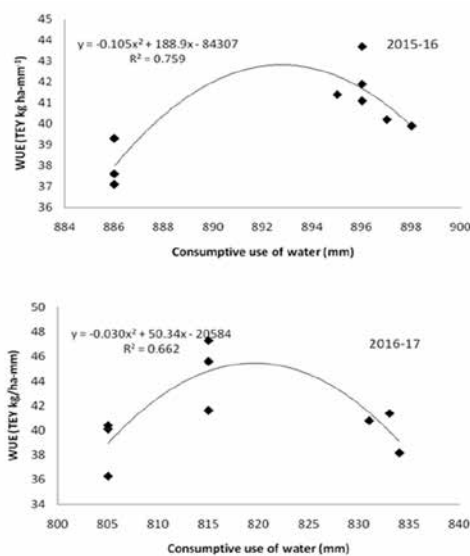


Fig. 1. Relationship between consumptive use of water and WUE in greater yam + maize intercropping system (Significant at $p=0.01$ in both the years)

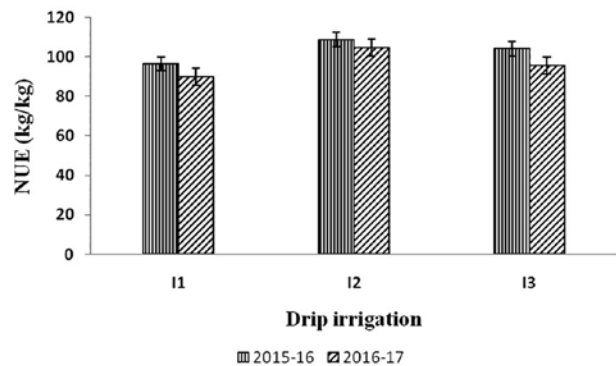


Fig. 2. Effect of drip irrigation levels on NUE [SEm±: 1.3 (2015-16) and 1.2 (2016-17); CD (P=0.05): 5.2 (2015-2016) and 4.8 (2016-2017)] in greater yam + maize intercropping system.

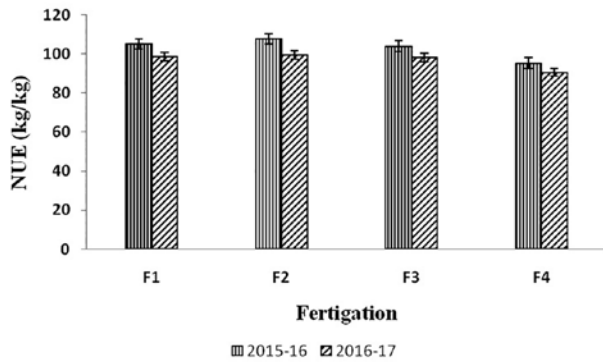


Fig. 3. Effect of fertilization levels on NUE [SEm±: 1.6 (2015-16) and 1.7 (2016-17); CD (P=0.05): 4.8 (2015-16) and 5.0 (2016-17)] in greater yam + maize intercropping system.

to greater TEY. The NUE had quadratic relationship with nutrient levels (Fig. 4). This indicated that addition of nutrients increased NUE up to F₂ and further addition of nutrients decreased NUE. At higher level of nutrients

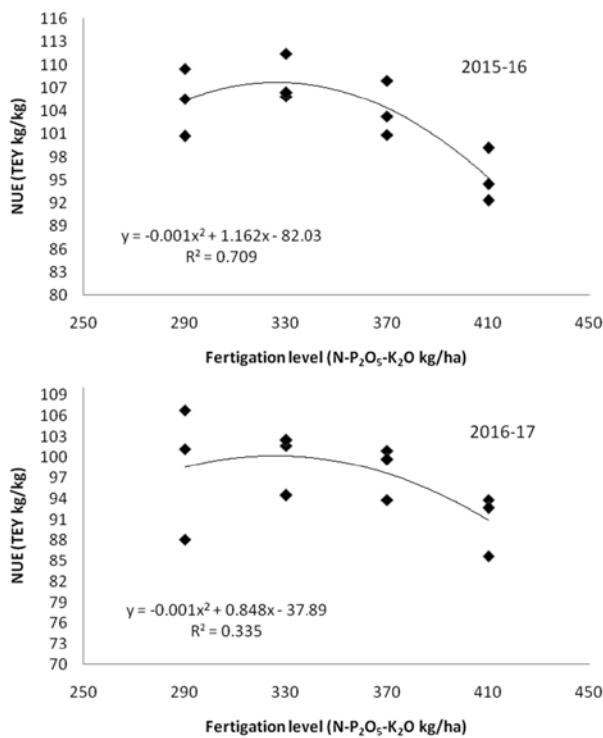


Fig. 4. Relationship between fertilization levels and NUE in greater yam + maize intercropping system (Significant at P=0.01 during 2015-16 and not significant during 2016-17)

application, yield increased at decreasing rate thereby NUE decreased. The interaction treatments I₂F₂ and I₂F₃ resulted in greater NUE (Fig.5). Application of same quantity of nutrients in soil and through drip irrigation revealed that application of nutrients along with drip irrigation at I₂ resulted in 16.5-19.0% greater NUE than

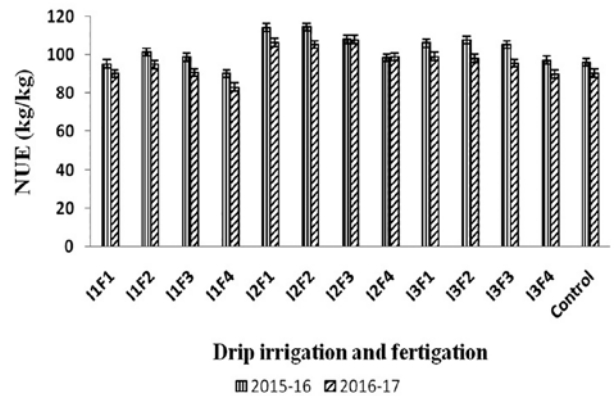


Fig. 5. Effect of drip irrigation and fertilization levels on NUE [SEm±: 2.8 and CD (P=0.05): 8.1 for both the years] in greater yam + maize intercropping system

soil application with the surface irrigation (control). The application of same quantity of nutrients along with drip irrigation at I₃ resulted in 8.6-12.2.0% greater NUE than soil application with the surface irrigation (control).

Thus, it can be concluded that the treatment I₂F₃ was at par with the treatment I₂F₄ in all the parameters studied. Hence, the treatment I₂F₃ (drip irrigation at 100% of CPE during 1-90 DAP+80% of CPE during 91-270 DAP along with fertigation of N-P₂O₅-K₂O 140-90-140 kg ha⁻¹) is recommended for greater yam + maize intercropping system considering greater TEY, WUE and NUE, and minimum water required per kg of TEY.

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Phenolic content and *in vitro* bioactivities of Chinese Potato (*Plectranthus rotundifolius*) tuber extracts

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Abstract

Plectranthus rotundifolius, commonly known as Chinese potato in India, is a perennial herbaceous plant of the Mint family *Lamiaceae* and is native to the tropical Africa. These are found to be rich in nutrients and have great medicinal properties. The tubers contain several secondary metabolites that are of therapeutic and pharmaceutical importance. The tubers in the dry and cooked form were analysed for the phenolic content and the *in vitro* bioactivities including antioxidant, anti-inflammatory and anti-diabetic activities by various biochemical assays. The tubers were also subjected to cooking to find out its effect on the phenolic content and antioxidant activities. The study revealed that cooked tubers have greater phenolic content and antioxidant, anti-inflammatory and antidiabetic activities than the raw tubers and it indicates that cooked form of tuber is better to consume than the raw tuber.

Keywords: Chinese potato, bioactivity, phenolics, flavonoids

Introduction

Roots and tuber crops play a substantial role in food security and nutrition. Most of the tuber crops are potential sources of bioactive phytochemicals including phenols and flavonoids (Farombi et al., 2000, Champagne et al., 2011). Apart from cassava and sweet potato, there are several minor root and tuber crops which are rich sources of bioactive phytochemicals and Chinese potato is one among them. Chinese potato (*Plectranthus rotundifolius* or *Solenostemon rotundifolius*) is a perennial herbaceous plant of the Mint family *Lamiaceae*. These plants are native to tropical Africa and are called in different names such as Native Potato, Country Potato, Hausa Potato or Sudan Potato. In India, these are known as Chinese Potato. The coleus potatoes contain reducing sugar, protein, crude fat and crude fibre (Anbuselvi et al., 2013) and are of great medicinal value and it lowers

the blood cholesterol as well as fends off the fungal and viral infections in humans. The mature tubers are used as a substitute for potatoes. There are several secondary metabolites present in these tubers with potential therapeutic and pharmaceutical applications. The leaves of these plants are used in traditional medicines for the treatment of dysentery, treatment of blood in urine, eye disorders etc. Several species of *Plectranthus* are used as folk medicine for skin irritations, antiseptics, vermicide and nausea (Narukawa et al., 2001).

The bioactivity of Chinese potato tubers is mostly due to the phenolic compounds present in them. These are soluble in polar organic solvents. In human perspective, dietary phenolics are useful to human health, possibly by acting as antioxidants, anticarcinogens and cardioprotective agents. The antioxidant activities of these compounds are responsible for the health effects including the

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prevention of certain cancers and coronary heart diseases (Pietta, 2000). Flavonoids are the phenolics substances that are widely found in the fruits and vegetables. Studies reveals that the ingestion of flavonoids reduces the risk of cardiovascular diseases, metabolic disorders, and certain cancers. These effects are due to the physiological activity of flavonoids in the reduction of the oxidative stress, inhibiting low-density lipoproteins oxidation and platelet aggregation and acting as vasodilators in blood vessels. The antioxidant activity of the tubers of *Plectranthus rotundifolius* was analysed by DPPH assay (Chen and Ho, 1995) and ABTS assay (Re et al., 1999). Phytochemical analysis was performed in the leaves of the *Plectranthus* species, *Plectranthus amboinicus*. The leaves of *P. amboinicus* are rich in phytochemicals and secondary metabolites such as steroids, tannins, flavonoids and alkaloids, which are probably responsible for its medicinal properties (Ware et al., 2019). The species of *Plectranthus* are known to possess anti-inflammatory activities also. *Plectranthus amboinicus* inhibits the pain induced by acetic acid and formalin and the inflammation caused by carrageenan. The *in vitro* anti-inflammatory activity of the extract was determined by the method of the inhibition of protein denaturation (Padmanabhan and Jangle, 2012; Elias et al., 1988). Manikandan et al., (2016) reported that *Plectranthus rotundifolius* showed the presence of different functional groups such as alcohol, phenols, amines, alkanes, aldehydes, carboxylic acid, isocyanides, alkynes, isocyanates, ketones, tertiary and primary alcohols and chloro compounds. Previous studies showed that extracts of *Plectranthus rotundifolius* possess *in vitro* anti-tumour activity (Singh et al., 2013), antioxidant activity (Sandhya et al., 2001) and acts as cancer chemopreventing agent (Nugraheni et al., 2011).

The processing techniques may affect the bioactivity of Chinese potato tubers, since phenolics are highly soluble in water and are sensitive to temperature, pH conditions etc. Since the tubers are largely used for edible purpose, it is important to understand the phenolic content and antioxidant activity of the cooked tubers. Hence, the objectives of the present study were to estimate the phenolics and flavonoid contents in Chinese potato tubers in relation to the *in vitro* antioxidant, anti-inflammatory, and antidiabetic activities and to study the effect of cooking on the phenolic content and its bioactivity.

Materials and Methods

Materials

The fresh tubers of Chinese potato were collected from the experimental farm of ICAR-CTCRI. Methanol (99.5%) and 2, 2'-diphenyl-1-picryl hydrazyl (DPPH) were purchased from Merck India Pvt. Ltd. (Mumbai, India). Gallic acid was procured from Sigma-Aldrich Corporation, St. Louis, MO, USA.

Preparation of tuber extract

The fresh Chinese potato tubers were washed thoroughly with running water to make free of the adhered soil and then peeled and sliced into equal pieces. About 200g of the tuber slices were lyophilized. Another 200g of the raw tuber was boiled in 300ml of water for about 25-30 min till the tubers were cooked. The cooked tuber was then transferred into a beaker, cooled and lyophilized. The raw and cooked tubers were then ground into a powder by using a mill (IKA all basic mill). Then, 5g each of the sample was weighed and then homogenised with methanol. It was then centrifuged, and the supernatant was transferred to a bottle. The process was repeated until the yellow colour of the supernatant was completely removed. The collected supernatants of both raw and cooked tubers were concentrated by using a Rotary flash evaporator (Buchi Multivapor). The residue after concentration was dissolved in methanol and transferred to vials and used for further analysis.

Estimation of total phenolic content

The total phenolic content of the tuber extract was determined by Folin-Ciocalteu assay using Gallic acid as standard (Malick and Singh, 1980). Briefly, to a test tube containing 0.5 ml of the extract, 2 ml of methanol and 0.5 ml of Folin-Ciocalteu reagent were added and is followed by the addition of 2 ml of 20% sodium carbonate (Na_2CO_3) solution. All the tubes were thoroughly shaken and covered with aluminium foil and were kept in dark for 1 hour. After the incubation period, the samples were centrifuged, and the supernatant was separated. The absorbance was measured at 765 nm using a spectrophotometer (Perkin Elmer, Lambda-25, Switzerland) with methanol as the blank. Triplicate analyses were performed for both the extracts. Gallic acid (trihydroxybenzoic acid) was used as the standard. The total phenolic content was expressed as milligrams of gallic acid equivalents (mg of GAE g^{-1} of sample) in the calibration curve.

Estimation of total flavonoid content

The total flavonoid content in the tuber extracts was determined by the aluminium chloride colorimetric method (Patel et al., 2010). To a test tube, 1.5 ml of the extract was taken and 1.5 ml of 5% AlCl_3 solution was added. It was mixed well, and the tubes were covered with aluminium foil and incubated for 60 min at room temperature. After incubation, the absorbance was measured at 420 nm against a mixture of 1.5 ml of methanol and 1.5 ml of AlCl_3 as the blank. Triplicate analyses were performed for both the raw and cooked extracts of the tuber. A calibration curve was plotted using Quercetin as the standard. The total flavonoid content was expressed as milligrams of quercetin equivalents (mg of quercetin g^{-1} of sample).

In vitro bioactivity studies

In vitro antioxidant activity

The antioxidant activity of the extracts was determined by the DPPH and ABTS assays. Two hundred μl of both the extracts were diluted to 2 ml using methanol and were used for the assays.

DPPH assay

The free radical scavenging capacity of the extracts of Chinese potato tubers was evaluated according to the method of Chen and Ho (1995) with slight modifications. Briefly, 0.5 mM DPPH solution was prepared by weighing 0.0197 g (19.7 mg) of DPPH (2,2-diphenyl-1-picryl hydrazyl) and making up to a volume of 100ml using methanol. From the diluted extracts of raw and cooked tubers, different concentration of the extracts ranging from 5 μl -160 μl (5,10,20,40, 80 and 160 μl) were taken in different test tubes and all the samples were made up to 2 ml with methanol. Then, 1 ml of DPPH solution was added to all the tubes. The contents were thoroughly mixed, covered and were kept in dark for 30 min at room temperature. The absorbance of the violet coloured solution was measured by using a uv-visible spectrophotometer (Perkin Elmer) at 517 nm against methanol as the blank. Gallic acid was used as the standard. The percentage (%) inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100 \dots \text{Eqn 1}$$

ABTS assay

The free radical scavenging activity of the extract was determined by ABTS radical cation decolourization assay using gallic acid as the standard (Wojdyło et al., 2007). Different concentrations of the diluted extracts of raw and cooked Chinese potato tubers (5 μl , 10 μl , 20 μl , 40 μl , 80 μl , and 160 μl) were taken in different test tubes and were made to a volume of 2 ml using methanol. To these tubes, 1 ml of ABTS solution was added and mixed well. All the tubes were covered with aluminium foil and kept in dark for 20 min at room temperature. The absorbance of the green coloured solution was measured spectrophotometrically at 734 nm against methanol as the blank. The % inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100 \dots \text{Eqn 2}$$

Anti-inflammatory activity: Inhibition of protein denaturation

The anti-inflammatory activity of the extracts was determined by the protocol described by Padmanabhan and Jangle (2012) with slight modifications. Different concentrations of the raw and cooked tuber extract (5 μl , 10 μl , 20 μl , 40 μl , and 80 μl) were taken in different test tubes and 0.2 ml of egg albumin was added to all the tubes. The volume of each test tube was made up to 3 ml by using 0.2 M phosphate buffer of pH 6.6. The tubes were well vortexed and then incubated in a boiling waterbath for 10-15 min which resulted in the protein denaturation. The tubes were then cooled to room temperature and the activity of each mixture was measured at 660 nm using a spectrophotometer with buffer as the blank. Aspirin (Acetylsalicylic acid) was used as the standard. The anti-inflammatory activity was determined as :

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100 \dots \text{Eqn 3}$$

Antidiabetic activity

The antidiabetic activity of the Chinese potato tuber extracts was determined by DNS (3,5-Dinitrosalicylic acid) method to determine the α -amylase inhibitory activity, by quantifying the reducing sugar (glucose equivalent) liberated under the assay condition (Thakkar and Patel, 2010; Chen et al., 2001). Different concentrations of the raw and cooked Chinese potato tuber extracts (5 μl , 10 μl , 20 μl , 40 μl , 80 μl , and 160 μl) were taken in test tubes and 250 μl of 0.02M sodium phosphate buffer (pH 6.9) containing α -amylase enzyme (240 U/mL) was added to each tube and then incubated for 20 min at 37°C. Then, 250 μl of 1% starch solution, prepared in 0.02 M sodium phosphate buffer was added to the test tubes. The tubes were shaken well and incubated for 15 min at 37°C. Then 1 ml of 1% dinitrosalicylic acid (DNS) was added to all the tubes and then incubated in a boiling water bath for 10 min. To the cooled mixture, 2 ml of distilled water was added and the absorbance was measured at 540nm using the phosphate buffer as blank. The % inhibition was determined as follows:

$$\% \text{ Inhibition} = \frac{(AC - AC_b) - (AS - AS_b)}{(AS - AS_b)} \times 100 \dots \text{Eqn 4}$$

Where, AC was the absorbance of control, AC_b was the absorbance of control blank, AS was the absorbance of the sample, and AS_b was the absorbance of the sample blank.

Results and Discussion

Total phenolic content

The total phenolic content in the raw tuber was found to be 1027 mg 100 g⁻¹ and that in the cooked tuber was 6068.8 mg 100g⁻¹ on fresh wt. basis (Table 1). It was found that the total phenolic content was higher in the cooked tubers and it was about 5 times greater than that in the raw tubers. According to Bhavne and Dasgupta (2019), the cooked sample of *Plectranthus amboinicus* exhibited higher phenolic content (10 mg GAE g⁻¹) than the raw sample (8 mg GAE g⁻¹). The results of the present study are in agreement with this report. The percent gain in the total phenol content during cooking might be due to the breakdown of tough cell walls and release of trapped phenolic compounds (Oboh et al., 2007). Navarre et al., (2010) assessed the targeted phytonutrients survival upon cooking of potatoes by different methods and noticed that the total phenolics, chlorogenic acids, flavonols and vitamin C did not significantly decrease after cooking by any of the methods. Cooking typically resulted in an increase in the recoverable amounts of the phenolic compounds. Supporting this finding is that antioxidant capacity also showed a corresponding increase. However, in another study by Gumul et al. (2017), loss in polyphenolic compounds after cooking was observed in the tubers of some of the potato varieties and it negatively influenced their antioxidant activity. But some exception was also observed in some other varieties, where antioxidant activity was not altered by cooking process, in spite of lowered level of total polyphenols, including flavonoids.

Potato nutrients and bioactive components appears to be influenced by cooking methods. Vinita and Punia (2018), reported a variety of effects such as destruction, release and structural transformations of the phytochemicals taking place during cooking process. The total phenolic content of the potato and carrot were found to be 25.23 and 19.14 mg GAE 100 g⁻¹ on fresh weight basis and the total flavonoid content were 18.71 and 12.27 mg GAE/100 g respectively. An increase of 83% in the total phenolic content was observed in the boiled potatoes and they reported that there is an overall increase in the TPC and TFC content of the cooked potato and carrot with a significant increase in antioxidant activity also. Bembem et al., (2013) also reported that TPC and TFC were less affected by cooking of potato tubers. The TPC increased in all the cooking processes and DPPH activity of the cooked tuber was higher compared to that of raw potato tuber. A study by Blessington et al., (2010) showed an increase in most of the individual phenolic compounds after baking, frying and microwave processing when compared to the uncooked tuber samples.

Total flavonoid content

The flavonoid content in the cooked Chinese potato tubers was 7072.8 mg 100 g⁻¹ and that of the raw tuber was 1400 mg 100 g⁻¹ on fresh wt. basis (Table 1). According to Murthy et al., (2018), the total flavonoid content in the *Plectranthus rotundifolius* tubers in the methanolic extract and was about 22.59 mg g⁻¹ and this agrees with our results. The flavonoid content was found to be higher in cooked tuber as compared to the raw tubers. In the work of Vinita and Punia (2018), the total flavonoid content was found to be increased for the cooked potato and carrot. According to Muhamad et al., (2019), the TPC and TFC in the ethanolic extract of *Plectranthus amboinicus* tubers was significantly higher in the boiled samples than in the control and was correlated with the antioxidant activity. This suggested that boiling can be used as a method to enhance the antioxidant activity. According to Bhavne and Dasgupta (2019), the cooked sample of *Plectranthus amboinicus* exhibited higher flavonoid content than that in the raw sample. In the cooked samples, the flavonoid content was found to be 12.6mg QEAC g⁻¹ and in the raw sample, it was 7.2 mg QEAC g⁻¹. All these studies suggest that cooking enhances the TPC, TFC and the antioxidant activity.

Table 1. Total phenolic and flavonoid contents in Chinese potato tuber

Sample	Total Phenolics (mg 100 g ⁻¹ of fresh tuber)	Total Flavonoids (mg 100 g ⁻¹ of fresh tuber)
Raw tuber extract	1027.0	1400.0
Cooked tuber extract	6068.8	7072.8

In vitro bioactivity studies

DPPH scavenging activity

DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging assay is the most widely used method for the screening of the antioxidant activity of the plant extracts (Kedare and Singh, 2011). The scavenging activity was expressed as percentage inhibition and IC₅₀. The Chinese potato tuber possessed significant radical scavenging activity in comparison to the standard, Gallic acid (Table 2). The cooked tuber extract exhibited significantly higher antioxidant activity towards DPPH radicals than the raw tuber extract. The inhibition of DPPH was 96.8±2.75% at a concentration of 160 µg ml⁻¹ for the cooked tubers and 91.4±2.70% in the case of raw tuber extract at the same concentration (Table 2). The percentage inhibition increased with increase in concentration of the extract. The results

Table 2. The DPPH radical scavenging activity of Chinese potato tuber extracts and Gallic acid

Sample	Concentration ($\mu\text{g ml}^{-1}$)	Percentage of Inhibition (%)	IC ₅₀ ($\mu\text{g ml}^{-1}$)
Gallic acid	2	13.9±1.01	7.87
	4	26.7±1.15	
	8	59.9±1.84	
	16	91.3±2.64	
Raw tuber extract	10	23.7±1.01	35.97
	20	40.2±1.44	
	40	67.8±2.14	
	80	79.1±2.19	
	160	91.4±2.70	
Cooked tuber extract	10	26.1±1.12	26.38
	20	42.8±1.72	
	40	74.1±1.88	
	80	84.7±2.21	
	160	96.8±2.75	

*Values are the mean of three replications \pm standard deviation

showed that these tuber extracts have very high potential as natural antioxidants. The IC₅₀ was also lower for the extracts of cooked sample indicating more activity. According to Murthy et al. (2018), the DPPH scavenging activity of the tubers of *Plectranthus rotundifolius* increased with increase in concentration and the greater ability to scavenge the free radical was shown by the methanolic extract with an EC₅₀ of 15.9 $\mu\text{g ml}^{-1}$. The methanolic extracts of *Plectranthus hadiensis* (Forssk). Schweinf. spreng showed higher antioxidant activity as compared to the standards viz., ascorbic acid and BHT (Butylated Hydroxy Toluene) (Menon et al., 2012). Bembem et al., (2013) reported that cooking increased the antioxidant activity of potato. According to them, the DPPH activity of the raw and processed samples ranged from 16.13% in raw potato to 32.48% in sauteed potato. According to Navarre et al. (2010), cooking resulted in a recoverable amount of phenolic compounds and thus showed increase in antioxidant activity. Bhawe and Dasgupta (2019) reported that the total phenolics, total flavonoids and

the DPPH scavenging activity are higher for the cooked sample of *Plectranthus amboinicus* when compared to that of the raw sample. Vinita and Punia (2018) reported that the DPPH scavenging activity of the cooked potato and carrot were higher than that in the raw potato and carrot.

ABTS⁺ Radical scavenging assay

ABTS radical scavenging assay of the tuber extract revealed that the raw tuber and cooked tuber showed percentage inhibition of 93.7±3.25% and 98.6±3.84%, respectively at a concentration of 160 $\mu\text{g ml}^{-1}$ and the IC₅₀ values were 14.47 and 4.73, respectively. The IC₅₀ value of the standard, gallic acid was found to be 1.83 (Table 3). From the above results, it was understood that the extract of the cooked tuber exhibited significantly higher antioxidant activity than the raw tuber and similar result were obtained for DPPH radical scavenging assay also. Bellumori et al., (2017) reported that the boiled pink and violet-fleshed potatoes showed the highest efficacy as radical scavengers in the ABTS test. There was a better antioxidant activity against ABTS in case of *Plectranthus stocksii* (Muniyandi et al., 2017).

Table 3. ABTS radical scavenging activity of Chinese potato tuber extract

Sample	Concentration ($\mu\text{g ml}^{-1}$)	Percentage Inhibition	IC ₅₀ ($\mu\text{g ml}^{-1}$)
Gallic acid	1.2	33.8±1.51	1.83
	1.6	43.5±1.62	
	2	51.6±2.11	
	2.4	62.8±2.81	
	2.8	82.4±3.10	
Raw tuber extract	10	31.1±1.32	14.47
	20	52.1±1.54	
	40	74.4±1.85	
	80	82±1.89	
	160	93.7±3.25	
Cooked tuber extract	10	33.4±1.48	4.73
	20	55.5±1.59	
	40	79.5±2.24	
	80	87.2±2.09	
	160	98.6±3.84	

*Values are the mean of three replications \pm standard deviation

Anti-inflammatory assay by protein denaturation

The method of anti-denaturation of egg albumin was chosen to evaluate the anti-inflammatory property of the Chinese potato tuber extract. The cooked tuber extract had better anti-inflammatory activity than the raw tuber extract and the percentage inhibition of the raw tuber and cooked tuber extracts were 90.5±2.76%

and $94.5 \pm 2.81\%$, respectively at a concentration of $80 \mu\text{g ml}^{-1}$ (Table 4). The IC_{50} values were found to be 22.97 and 19.44 for extracts of raw and cooked tuber, respectively.

Table 4. Anti-inflammatory activity of the Chinese potato tuber extract

Sample	Concentration ($\mu\text{g ml}^{-1}$)	Percentage Inhibition	IC_{50} ($\mu\text{g ml}^{-1}$)
Aspirin	5	25.1 ± 1.15	16.21
	10	41.2 ± 1.52	
	20	65.0 ± 2.15	
	40	87.4 ± 2.52	
	80	97.1 ± 3.11	
Raw tuber	5	20.0 ± 1.12	22.97
	10	35.2 ± 1.81	
	20	60.0 ± 2.08	
	40	79.1 ± 2.13	
	80	90.5 ± 2.76	
Cooked tuber	5	22.1 ± 1.12	19.44
	10	40.2 ± 1.49	
	20	61.5 ± 2.03	
	40	82.6 ± 2.31	
	80	94.5 ± 2.81	

*Values are the mean of three replications \pm standard deviation

Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation (Sadique et al., 1989). The methanolic extract of *Coleus forskohlii* showed good activity towards the BSA anti-denaturation assay (Menon and Latha, 2011). When BSA is heated, it undergoes denaturation and expresses antigens associated with type III hypersensitive reaction related to disease like serum sickness. Several species of *Plectranthus* are known to possess the anti-inflammatory activities.

Antidiabetic assay: Inhibition of α -amylase

The tuber extracts exhibited a dose dependent inhibition of α -amylase activity and the percentage inhibition increased with increase in the concentration of the extract. The percentage of inhibition shown by the raw tuber and cooked tuber was $93.0 \pm 3.11\%$ and $96.7 \pm 2.11\%$, respectively at a concentration of $80 \mu\text{g ml}^{-1}$ indicating the higher inhibitory activity of cooked tuber extract against the α -amylase compared to the raw tuber extract (Table 5). The IC_{50} values of the raw and cooked tubers were found to be 23.01 and 16.20, respectively. Prathibha et al., (1995) reported that the coleus tuber possessed high anti-amylase activity. When the tubers were processed by the pressure cooking there was a significant reduction or complete elimination in the inhibitory activity.

Table 5. Inhibition of α -amylase by Chinese potato tuber extract

Sample	Concentration ($\mu\text{g ml}^{-1}$)	Percentage Inhibition	IC_{50} ($\mu\text{g ml}^{-1}$)
Raw tuber	5	21.1 ± 1.13	23.01
	10	35.3 ± 1.81	
	20	57.1 ± 1.79	
	40	79.5 ± 2.13	
	80	93.0 ± 3.11	
Cooked tuber	5	26.1 ± 1.18	16.20
	10	41.4 ± 1.50	
	20	65.8 ± 2.13	
	40	84.1 ± 1.91	
	80	96.7 ± 2.11	

*Values are the mean of three replications \pm standard deviation

Conclusions

There are several secondary metabolites present in Chinese potato (*Plectranthus rotundifolius*) tubers with potential therapeutic and pharmaceutical applications. The bioactivity of the tubers is mostly due to the phenolic compounds present in them. Since the tubers are largely used for edible purpose, it is important to understand the phenolic content and antioxidant activity of the cooked tubers. The total phenolic content and total flavonoid content was higher for the cooked Chinese potato tubers compared to the raw tubers. Also, the antioxidant assays including DPPH and ABTS assays performed revealed that the cooked tuber contains greater activity than the raw tuber. Anti-inflammatory and antidiabetic assays also revealed that cooked tuber was having greater activity than raw tuber. The study reveals the importance of including this tuber in our everyday diet.

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Integrated weed management in elephant foot yam cv. Gajendra

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Abstract

The present investigation was undertaken at Shaheed Gundadhur College of Agriculture and Research Station, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Jagdalpur, Chhattisgarh during 2019 and 2020 to assess the effect of different weed management practices in *Amorphophallus* cv. Gajendra. The experiment was laid out in RBD with three replications with eight different treatments based on the individual or combination of pre emergence herbicide, post emergence herbicides, hand weeding, ground cover and control. Among the treatments T₅ (Post-emergence herbicide at 30, 60 and 90 DAP) recorded the highest WCE (89.66%) followed by T₄ (Hand weeding at 45 DAP + Post-emergence herbicide at 90 DAP) 88.92%. Significantly higher yield, corm weight per plant and per hectare were recorded in T₅ treatment (Post-emergence herbicide at 30, 60 and 90 DAP) followed by T₁ (Pre-emergence herbicide (1DAP) + Post-emergence herbicide at 45 and 90 DAP).

Keywords: Elephant foot yam, weed, herbicide, yield, Economics.

Introduction

Elephant foot yam [*Amorphophallus paeoniifolius* (Dennst.)] is a tropical tuberous vegetable crop grown in all India originated from South-East Asia. In India it is known as Suran or jimikand. In Chhattisgarh, it is cultivated in *Kharif* season for edible corms. The area and production of elephant foot yam in Chhattisgarh is 3518 ha and 40.83 lakh metric tons, respectively. Elephant foot yam is a highly nutritive vegetable (Gopalan et al., 1999). Corm are cooked as vegetables, boiled or baked. Even the stem portion of the plant is used for preparing *badi* in Chhattisgarh, a value added product of *colocasia* stem mixed with black lentil which can be stored in dried form. Because of its medicinal properties, corm is used in curing piles, dysentery, and acute rheumatism. Elephant foot yam, being a *Kharif* and long duration crop is liable to be highly infested with weeds which is

extremely hazardous both in terms of crop health as well as productivity. It has been well established that the yield loss due to weeds is quite higher (45%) than the pests (30%) and diseases (20%) (Nedunchezhiyan et al., 2018). Sometimes weed roots penetrate into the underground storage organs of tuber crops and reduce the quality of produce (Suresh et al., 2019). Weeds compete for all available resources both below (water, nutrients, space) and above ground (space, light) and thereby reduce the crop growth and yield (Suresh et al., 2020). Yield losses of crops because of weed competition are estimated to be 40-90% in cereals, 50-60% in legumes, 50-53% in oilseeds, and 65-91% in root and tuber crops (Ado, 2007). Manual weeding in a crop is a labour-intensive process and other cultural practices are also affected. There is a need for technologies to make hand weeding more efficient to achieve acceptable weed control in

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production fields. Improvement in chemical control of weeds and the introduction of new weed management technologies to reduce cost of production is very much needed in elephant foot yam as it is a commercial tuber crop growing all over Chhattisgarh state. In this regard, present study was undertaken to find out the most efficient and economic integrated weed management practices in Elephant Foot Yam.

Materials and Methods

An experiment was carried out at the experimental field of All India Coordinated Research Project on Tuber Crops (AICRP TC), Saheed Gundadhur College of Agriculture and Research Station, Jagdampur, during *Kharif* 2019 and 2020. The experiment was laid out in a randomized block design with three replications and eight treatments *viz.*, T₁: Pre-emergence herbicide (Pendimethalin 30% EC) 1 day after planting (DAP) + Post emergence herbicide (Glyphosate 41% SL) at 45 and 90 DAP, T₂: Pre-emergence herbicide (1DAP) + hand weeding at 45 and 90 DAP, T₃: Raising green manure crop pea in interspaces along with planting and incorporation 45-60 DAP + Post emergence herbicide at 90 DAP, T₄: Hand weeding at 45 DAP + Post emergence herbicide at 90 DAP, T₅: Post emergence herbicide at 30, 60 and 90 DAP, T₆: Weed control ground cover, T₇: Hand weeding at 30, 60 and 90 DAP and T₈: Control no weeding. Planting distance for row to row and plant to plant was kept as 90×90 cm in a plot size of 4.5×4.5 m. The healthy cut corm pieces or whole corms of the *Amorphophallus* variety, 'Gajendra', weighing 500 g and treated with Bavistin (fungicide 2.5 g @ per litre of water) before planting were used as planting materials. Pendimethalin used as pre-emergence herbicide and was applied one day after the planting of corms in optimal

soil moisture condition. Glyphosate was used as post-emergence herbicide and applied in the plots as per treatments. To protect the main crop, herbicides were applied without drift on elephant foot yam plants with a manually operated knapsack sprayer with a flat-fan nozzle attached to a hood using a spray volume of 500 lit ha⁻¹. Paddy straw was used as weed control ground cover and immediately covered after planting.

All the recommended cultural practices were taken to grow a healthy crop. Data were recorded on five randomly selected plants with respect to characters *viz.*, plant height (3 months after planting (MAP) & 5 MAP), girth of pseudo stem (3 MAP & 5 MAP), canopy Spread (3 MAP & 5 MAP), leaf area (3 MAP & 5 MAP), total yield, yield per plant, weed density, weed control efficiency, and dry weight. The data were recorded for growth, yield and economics and statistically analyzed. Weed control efficiency (WCE) was calculated on the basis of dry matter production of weeds. Analysis of variance was done as per Panse and Sukhtme (1967). As per the design of experiment, the data on plant growth and weed parameters over the year were pooled and analyzed using PB tools, IRRI. Treatment means were compared using Turkey's studentized range (HSD) at 5% probabilities.

Results and Discussion

The analysis of variance revealed that all the characters measured were significantly different under the treatments (Table 1). During both the seasons the weed species were recorded (Table 2). Among broad leaves weeds *Spilanthus acmella*, *Celosia argentea*, *Commelina benghalensis*, *Euphorbia geniculata* were the major weeds. Grasses and sedges such as *Setaria glauca*, *Cyperus rotundus*, *Digitaria sanguinalis*, *Eleusine indica*, and *Echinochloa colona* were also dominant in the experimental plot.

Table 1. Analysis of variance for tuber yield and other characters of elephant foot yam

Sl. No.	Character	Mean Sums of Square		
		Replication	Treatment	Error
1	Plant height (cm) 3 MAP	1.5126	166.59**	7.84
2	Plant height (cm) 5 MAP	20.22	265.91**	6.15
3	Canopy Spread (cm) 3 MAP	9.60	106.05**	12.25
4	Canopy Spread (cm) 5 MAP	11.63	97.28**	10.12
5	Leaf area (cm) ² 3 MAP	1155659.35	19775789.11**	4152209.95
6	Leaf area (cm) ² 5 MAP	3008326.86	29173374.23**	6216478.04
7	Girth of Pseudo stem (cm) 3 MAP	0.98	3.80*	0.42
8	Girth of Pseudo stem (cm) 5 MAP	2.70	4.18*	0.46
9	Weed Density	0.10	1002.06**	0.84
10	Weed control efficiency	3.54	2668.50**	2.08
11	Dry Weight	6.63	3123.89**	23.45
12	Corm yield (t ha ⁻¹)	37.15	50.99**	4.30

*Significant at 5%; **Significant at 1%; MAP: Months after planting

Table 2. Weed Flora observed during 2018-19 and 2019-20 in the elephant food yam experimental field

Sl. No.	Weed Species	Weeds name
1	Broad leaved weed	Spilanthes acmella, Celosia argentea, Commelina benghalensis, Euphorbia geniculata.
2	Grasses and Sedges Weed	Setaria gluaca, Cyperus rotundus, Digitaria sanguinalis, Eleusine indica, Echinochloa colona and others

Minimum weed density and dry weight were recorded in the treatment T_5 with post emergence herbicide (glyphosate) at 30, 60 and 90 DAP followed by the treatment T_2 i.e., pre-emergence herbicide (1DAP) + hand weeding at 45 and 90 DAP. Herbicide application at specific intervals did not allow the weeds to emerge. Among the eight different treatments of weed management, the weed control efficiency ranged from 73.76-89.66 % (Table 3). The maximum WCE of 89.66% was observed in the treatment T_5 , i.e., post emergence herbicide (Glyphosate) at 30, 60 and 90 DAP (89.66 %), followed by T_4 , (WCE-88.92%), i.e., raising green manure cow pea in inter space along with planting and incorporation at 45-60 DAP followed by glyphosate application at 90 DAP because of the lower weed density. The weed density and dry weight of the weed were maximum in the weedy check (control) (Table 3). Singh et al., (2020) also reported that maximum WCE was seen in EFY when glyphosate was applied, or hand weeding was done. Singh et al., (2018) observed that combination of pre and post emergence application of herbicide was effective for reducing the number of weeds as compared

to the control. Similar results were also reported by Sekhar et al., (2017) and Singh et al., (2020) in elephant foot yam.

Plant growth parameters such as plant height, leaf area, girth of pseudo stem and canopy spread were significantly influenced by the different weed control treatments (Tab. 4). All the treatments resulted in significantly taller plants, maximum leaf area, wide girth of pseudo stem and canopy spread than the control. Among all the treatments, at 3 MAP, maximum plant height of 83.11 cm was recorded with weed control ground cover with paddy straw (T_6) followed by post emergence at 30, 60 and 90 DAP (T_5) with the height of 81.23 cm. The maximum leaf area and girth of pseudo stem were recorded with pre-emergence herbicide (Pendimethaline 30% EC) 1 DAP followed by glyphosate application at 45 and 90 DAP (T_1). Maximum canopy spread was recorded with pre-emergence herbicide (1 DAP) followed by hand weeding at 45 and 90 DAP (T_2). Briefly, in the initial crop growth stage, the four treatments, viz., weed control ground cover with paddy straw, application of

Table 3. Effect of treatments on weed density, dry weight and weed control efficiency of elephant foot yam cv. Gajendra (pooled analysis of 2018-19 and 2019-20)

Treatment	Weed Density [No. (m ²) ⁻¹]	Dry Weight [g (m ²) ⁻¹]	Weed control efficiency (%)
T_1 : Pre-emergence herbicide (1DAP)+Post-emergence herbicide at 45 and 90 DAP	15.09 ^b	29.66 ^b	85.77 ^a
T_2 : Pre-emergence herbicide (1DAP)+hand weeding at 45 and 90 DAP	7.42 ^{ef}	7.54 ^c	85.63 ^a
T_3 : Raising green manure crop pea in interspaces along with planting and incorporation 45-60 DAP+Post-emergence herbicide at 90 DAP	10.47 ^{cd}	9.26 ^c	75.52 ^{bc}
T_4 : Hand weeding at 45 DAP+Post-emergence herbicide at 90 DAP	8.52 ^{def}	30.25 ^b	88.92 ^a
T_5 : Post-emergence herbicide at 30, 60 and 90 DAP	6.48 ^f	8.87 ^c	89.66 ^a
T_6 : Weed control ground cover (Paddy straw)	12.80 ^{bc}	37.50 ^b	79.35 ^b
T_7 : Hand weeding at 30, 60 and 90 DAP	9.47 ^{de}	10.16 ^c	73.76 ^c
T_8 : Control (No weeding)	61.11 ^a	103.88 ^a	0.00 ^d
HSD ($\alpha=0.05$)	2.64	13.95	4.15
CV (%)	5.59	16.34	1.99

* Values in each column with the same alphabets in the superscripts do not differ significantly

post-emergence herbicide thrice at 30, 60 and 90 DAP, pre-emergence herbicide (Pendimethaline 30% EC) 1 DAP followed by glyphosate at 45 and 90 DAP and pre-emergence herbicide (1 DAP) followed by hand weeding at 45 and 90 DAP suppressed weed growth and gave similar effect. Similar report observed by Singh et al., (2020) and Sekhar et al., (2020) in elephant foot yam.

At five months after planting, maximum plant height and leaf area were observed with weed control ground cover with paddy straw (T_6). Similar findings were also observed by Sekhar et al., (2017) when black polythene mulch was used where yield characters such as height, diameter, volume of corm increased. Girth of pseudo

stem recorded maximum with post-emergence herbicide at 30, 60 and 90 DAP (T_5). The canopy spread was highest with pre-emergence herbicide (1 DAP) followed by hand weeding at 45 and 90 DAP (T_2) (Table 4).

Yield was directly influenced by crop growth and crop growth of EFY was influenced by different treatments. Lower corm yield was recorded with weedy check (25.22 t ha^{-1}) as no control measure were adopted to control the weed in this treatment. Treatment with post-emergence herbicide at 30, 60 and 90 DAP has given higher yield (38.12 t ha^{-1}) which was at par with pre-emergence herbicide 1 DAP followed by glyphosate at 45 and 90 DAP (Table 5). This could be due to the high

Table 4. Plant growth parameters as affected by integrated weed management treatments in elephant foot yam cv. Gajendra (pooled analysis of 2018-19 and 2019-20)

Treatment	Plant height (cm)		Leaf area (cm^2)		Girth of Pseudo stem (cm)		Canopy Spread (cm)	
	3 MAP	5 MAP	3 MAP	5 MAP	3 MAP	5 MAP	3 MAP	5 MAP
T_1	78.80 ^{ab}	86.03 ^{bc}	11086.15 ^a	12760.05 ^{ab}	11.93 ^a	14.19 ^{ab}	91.56 ^{abc}	102.92 ^{ab}
T_2	63.48 ^d	71.48 ^d	9330.89 ^{ab}	10475.49 ^{abc}	10.86 ^{abc}	13.51 ^{abc}	96.71 ^a	107.33 ^a
T_3	66.93 ^{cd}	73.16 ^d	9454.55 ^{ab}	10348.81 ^{abc}	9.48 ^c	12.09 ^c	80.00 ^d	91.73 ^c
T_4	73.83 ^{bc}	81.58 ^c	7451.12 ^{abc}	9191.08 ^{abc}	9.17 ^c	11.67 ^c	82.36 ^{cd}	93.05 ^c
T_5	81.23 ^{ab}	92.15 ^{ab}	8121.40 ^{abc}	9423.91 ^{abc}	11.75 ^{ab}	14.75 ^a	93.34 ^{ab}	103.28 ^{ab}
T_6	83.11 ^a	94.48 ^a	8780.03 ^{abc}	13534.12 ^a	9.89 ^{bc}	12.52 ^{bc}	82.74 ^{cd}	93.70 ^c
T_7	73.53 ^{bc}	80.43 ^c	4991.50 ^{bc}	6001.82 ^{bc}	9.38 ^c	12.37 ^{bc}	90.05 ^{abcd}	100.78 ^{abc}
T_8	65.42 ^d	69.31 ^d	3196.70 ^c	4288.60 ^c	9.36 ^c	11.57 ^c	86.33 ^{bcd}	96.73 ^{bc}
HSD ($\alpha=0.05$)	8.06	7.14	5870.90	7183.52	1.86	1.96	10.08	9.16
CV (%)	3.81	3.06	26.12	26.24	6.32	5.30	3.60	3.22

*MAP: Months after planting

** Values in each column with the same alphabets in the superscripts do not differ significantly

Table 5. Yield and economics of elephant foot yam under different weed management treatments (pooled analysis of 2018-19 and 2019-20)

Treatment	Corm yield (t ha^{-1})	Gross return	Net return	B:C Ratio
T_1	36.89 ^{ab}	737800	454351	2.60
T_2	29.42 ^{cd}	588400	304711	2.07
T_3	30.11 ^{cd}	602200	317351	2.11
T_4	31.76 ^{bc}	635200	351511	2.24
T_5	38.12 ^a	762400	478391	2.68
T_6	32.57 ^{abc}	651400	369691	2.31
T_7	30.91 ^{cd}	618200	332851	2.17
T_8	25.22 ^d	504400	223701	1.80
HSD ($\alpha=0.05$)	5.97	-	-	-
CV (%)	6.51	-	-	-

weed control efficiency and lower weed density that boosted crop growth and yield attributes and resulted in higher corm yield. Sekhar et al., (2017) reported best weed management practices in elephant foot yam was mulching with black polythene and other better treatments were combination of pre-emergence application of oxyfluorfen 0.2 kg ha⁻¹ + manual weeding at 75 DAP or post-emergence application of glyphosate 0.8 kg ha⁻¹ + manual weeding at 75 DAP.

Among all the different weed management practices adopted, significantly maximum B:C ratio was recorded with post-emergence herbicide at 30, 60 and 90 DAP with maximum gross return and net return. It was followed by pre-emergence herbicide 1 DAP followed by glyphosate at 45 and 90 DAP, due to higher corm yield and saving of labour wages in comparison to hand weeding. A maximum B:C ratio was reported in hand weeding at 30, 60 and 90 DAP with maximum corm yield of 41.54 t ha⁻¹ (Singh et al., 2020). In another study, the B:C ratio was highest when pendimethilin + Quizalofop-p-ethyl at 40 DAS was used to control weeds (Singh et al., 2018).

Conclusion

The application of pre-emergence and post-emergence herbicide in wide spaced crops like elephant foot yam is an efficient and economic method for weed control and it also saves time. It may be used as an alternative way of weed control where labour availability for agricultural operations is a problem.

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Time series modelling of monthly rainfall in southern Kerala

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Abstract

This paper aimed to fit SARIMA model based on Box-Jenkins methodology to the time series data corresponds to monthly rainfall in three agro climatic regions *viz.*, Regional Agricultural Research Station (RARS) Vellayani, RARS Kumarakom and Cardamom Research Station (CRS), Pampadumpara representing different regions of Southern part of Kerala. The empirical model gave a picture of climate change scenario happened in both temporal and regional wise. The SARIMA model was fitted to monthly rainfall for all the regions Vellayani, Kumarakom, and Pampadumpara using the data for 31 years from 1991 to 2021. The best identified SARIMA models for rainfall were ARIMA (1, 0, 0) (0, 1, 1)₁₂, ARIMA (0, 0, 0) (0, 1, 1)₁₂ and ARIMA (0, 0, 0) (0, 1, 1)₁₂. The model parameters were obtained by using maximum likelihood method and the best model were selected using Akaike Information Criteria (AIC), Bayesian Information Criteria (BIC) and Hannan-quinn coefficient. The adequacy of the check of the selected models confirmed that the selected models were free from autocorrelation and the residuals are normally distributed.

Keywords: Seasonal Autoregressive Integrated Moving Average, Regional Agricultural Research station, Akaike Information Criteria, Bayesian Information Criteria

Introduction

Indian agriculture has been historically explained as a gamble with monsoon because agricultural activity in most parts of the country depends mainly on monsoon. India is heavily dependent on South-West monsoon (June-September) for most of its annual rainfall. The Kerala state, known as “Gateway of monsoon in India” is one of the unique regions in the humid tropical monsoon climate which receives high solar radiation and warm temperature throughout the year since it is at a short distance away from the equator. Unimodal and bimodal distribution of rainfall with undulating topography, varied soil types and sharp changes in physiography (below msl to 2500 m above msl), together with 44 rivers, many freshwater lakes and estuarine backwaters give rise to contrasting ecological units congenial for

high biological activity, contribute for its rich biodiversity in Kerala. The principal rainy seasons in Kerala are the South-west monsoon (June-September) and the North-East monsoon (October-November). The pre-monsoon months (March-May) are characterized by major thunderstorm activity in the state and winter months (December-January) are marked by low clouding and low rainfall season. Time series modeling of weather parameters especially rainfall will describe the overall variations noticed in the pattern and predict the future distributional behavior.

Materials and Methods

The time series approach used in this study is based on ARIMA - Box-Jenkins methodology. ARIMA uses the autocorrelation relationship exists in the data set for model development and forecasting.

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Stationarity and differencing

Stationary time series data is characterized by its unique nature of time independency of its various properties like mean, variance. A time series $\{x_t\}$ is said to be strictly stationary, if the joint probability distribution of observations $(x_t, x_{t+1}, \dots, x_{t+n})$ is exactly same as the joint probability distribution of observations $(x_{t+h}, x_{t+h+1}, \dots, x_{t+h+n})$ for every point $(t, t + 1, \dots, t + n)$ where h is the time space. The process $\{x_t\}$ is said to be weakly stationary, if it has a constant mean, finite variance and its auto-covariance function $\gamma(t,s)$ depends only on the time lag $|t-s|$. There are many ways in which a time series fails to be stationary, and those are said to be non-stationary time series. Modelling of a non-stationary data will have no sense, so data should be stationary before fitting a model. By the method of differencing non-stationary data can be converted to stationary.

Differencing will stabilize the mean of the time series by eliminating or reducing trend and seasonality. Differenced series will be the change between consecutive observations. Ordinary differencing and seasonal differencing are the common ways to eliminate non-stationarity in the data.

First order differenced series:

$$y'_t = y_t - y_{t-1}$$

Second order differenced series:

$$y''_t = y'_t - y'_{t-1}$$

$$y''_t = y_t - 2y_{t-1} + y_{t-2}$$

Usually, ordinary second order differencing will be enough to make the data stationary. Sometimes seasonal differencing will also be found necessary to remove non-stationarity. This is nothing but difference between consecutive observations in the same season denoted as $y'_t = y_t - y_{t-m}$, where m is seasonal term.

Unit root test

The modern technique used to detect stationarity of the time series data is through unit root test. Several unit roots tests are available such as Augmented Dickey Fuller test (ADF), Kwiatkowski-Phillips-Schmidt-Shin(KPSS) test etc. In this study ADF test is used for detecting the stationarity.

The null hypothesis and the alternative hypothesis for ADF test was:

H_0 : Presence of unit root indicating time series data is non stationary.

H_1 : Absence of unit root indicating time series data is stationary.

The test statistic for ADF test is defined as follows:

$$DE_t = \frac{\gamma}{SE(\gamma)}$$

If DE_t was found greater than critical value or p -value less than 0.05, then H_0 was rejected.

Autocorrelation and Partial autocorrelation functions (ACF and PACF)

The classical method used to determine whether data is stationary or not is by analyzing the nature of ACF and PACF plots. These plots graphically summarize the strength of association between observations in present time with its previous period.

Auto correlation is the correlation between observations of a variable taken at different time points. Auto Correlation Function (ACF) plots are widely for checking randomness in a data set. This randomness is ascertained by computing auto correlations for data values at varying time lags. Partial Auto Correlation Function (PACF) of $\{Z_t\}$ is a partial correlation coefficient between $\{Z_t\}$ and $\{Z_{t-k}\}$ by fixing the effect of others. PACF of order k is the correlation coefficient between $\{Z_t\}$ and a suitable linear combination of Z_t, Z_{t-1}, \dots . ACF and PACF plots are drawn by considering correlation coefficients on the y-axis with number of lags in the x-axis.

Autoregressive model (AR Model)

In an autoregressive (AR) Model, each value in a series should be a linear function of the preceding value or values. In a first-order autoregressive process, only the single preceding value is used or in a second-order process, the two preceding values are used, and so on. These processes are commonly indicated by the notation AR(p) or ARIMA(p-0-0), where the number in parentheses indicates the order.

AR model of order p can be written as:

$$y_t = c + \phi_1 y_{t-1} + \phi_2 y_{t-2} + \dots + \phi_p y_{t-p} + \epsilon_t$$

Moving average model (MA Model)

In this model, instead of considering past values of forecast variables past values of forecast errors are considered in the regression equation. In a moving-average process, each value is determined by the weighted average of the current disturbance and one or more previous disturbances. The order of the moving-average process specifies how many previous disturbances are averaged into the new value.

MA model of order q can be written as

$$y_t = c + \epsilon_t + \theta_1 \epsilon_{t-1} + \theta_2 \epsilon_{t-2} + \dots + \theta_q \epsilon_{t-q}$$

Non-seasonal ARIMA Model

Combination of AR and MA models along with order of integration or difference will form an Autoregressive Integrated Moving Average (ARIMA) model.

The full model will be in the form:

$$y'_t = c + \phi_1 y'_{t-1} + \dots + \phi_p y'_{t-p} + \theta_1 \epsilon_{t-1} + \dots + \theta_q \epsilon_{t-q} + \epsilon_t$$

Where y'_t the differenced series and right-hand side contain predictors of lagged values of y_t and ε_t (residual term).

The form of ARIMA (p,d,q) can also be written as,

$$\phi + (B) (1-B)^d Z_t = \theta(B) \varepsilon_t$$

Where ϕ – Coefficient of non-seasonal AR component

B – Backshift operator

θ – Coefficient of non-seasonal MA component

This can be notated simply as ARIMA (p, d, q)

Where p – Order of autoregressive part

d – Order of integration

q – Order of moving average part

Seasonal ARIMA (SARIMA) Model

The SARIMA model is formed by including a seasonal component to the ARIMA model. It can be represented as ARIMA (p, d, q) (P, D, Q) in which p and q are non-seasonal autoregressive and moving average parameters, P and Q are the seasonal autoregressive and moving average parameters, respectively. The two other parameters, d and D, are non-seasonal and seasonal differencing respectively, used to make the series stationary.

The form of ARIMA (p,d,q) × (P,D,Q) has the following form,

$$\phi_p(B)\Phi_p(B^s)\nabla^d\nabla^D_s Z_t = \theta_q(B)\Theta_q(B^s)\varepsilon_t$$

Where ϕ – Coefficient of seasonal AR component

Φ – Coefficient of seasonal MA component

To obtain the ARIMA model by the Box-Jenkins methodology, there are three steps that must be considered which are identification, parameter estimation, and diagnostic checking (goodness of fit test).

Identification

In this step, three integers p, d, and q and P, D, Q representing respectively the number of autoregressive orders, the number of differencing orders, and the number of moving-average orders of both non-seasonal and seasonal part of ARIMA model are determined. Stationarity check of the data set reveals the nature of order of integration included in the model. It can be done by using classical methods involving autocorrelation functions (ACF) and partial autocorrelation functions (PACF) plots and modern methods such as Augmented Dickey Fuller test (ADF) (Saha et al., 2016).

Estimation of parameters

After estimating order of the model next step is to determine the parameters such as c, $\phi_1, \dots, \phi_p, \theta_1, \dots, \theta_q$ etc. The parameters can be estimated using a function

minimization algorithm, either minimize the sums of squared residuals or maximize the likelihood (probability) of the observed series. To compute the sums of squares (SS) of the residuals, the approximate maximum likelihood method (MLE) is chosen, as this method is the fastest and can be used for very large data sets. For ARIMA model, MLE was similar to least square estimate which is based on minimizing the function $\sum_t \varepsilon_t^2$. Since the ARIMA model is much complicated to estimate the regression models, certain model selection criteria were used by most of the software including open-source software Gretl, which is used in this study.

Information criteria

Model selection was done based on Akaike’s Information Criteria (AIC), Bayesian Information Criteria (BIC) and Hannan-Quinn Criteria (HQIC).

AIC is useful in selecting predictors for regression as well as determining order of an ARIMA model. It can be written as

$$AIC = -2 \log(L) + 2 (P+Q+K+1)$$

Where, L was the maximum likelihood function and last term represent the number of estimated parameters, in which K=0 if c=0 and K=1 if c≠0. (Akaike, 1974).

For ARIMA model, corrected AIC denoted as AIC_c can be written as:

$$AIC_c = \left(\frac{2n}{n-k-1}\right) K - 2\ln[L]$$

Where, n was the number of observations

BIC or Schwarz information criteria (SIC)

$$SIC = \ln(n)K - 2\ln(L) \text{ (Schwartz, 1978)}$$

$$HQIC = 2\ln[\ln(n)]K - 2\ln(L) \text{ (Hannan and Quin, 1979)}$$

Validation of the model

Once the preferred model is identified, standardized residuals should be analyzed. According to our model assumption, observations are normally distributed and thus, the standardized residuals should be standard normally distributed. Now, if a model was found to be not good enough, then errors will no longer remain uncorrelated and like a time series depends on its past values, the errors will remain uncorrelated as well. So, model validation can be made by analyzing the nature of residuals in terms of autocorrelation and normality.

Residual Analysis

When a model has been identified as best fit to a time series, it is inevitable to check that whether the selected model provides an adequate representation of the data. This is usually done by looking at the residuals. For a good model, residuals are stationary and uncorrelated, and a model validation usually consists of plotting the

residuals in various methods. Another way is by detecting whether residuals follow a normal distribution, and if so, the model selected will be good.

Ljung-Box Test

The test was used to determine whether the autocorrelations for the errors or residuals are non-zero (Modified Box-Pierce statistic) (Sallehuddin et al., 2007; Kane and Yusof, 2013)

The null and alternate hypothesis of the test are given below:

$$H_0: \text{The errors are uncorrelated}$$

$$H_1: \text{The errors are correlated.}$$

The test statistic was:

$$Q_m = n(n+2) \sum_{k=1}^m \frac{Y_k^2}{n-k}$$

Where n was the number of observations, Y_k was the autocorrelation between residuals with lag k and m total number of lags. The statistic Q_m had a finite sample distribution that was much closer to that of χ^2 (m-p-q). The procedure was to reject the null hypothesis of uncorrelated residuals, if the computed value of Q_m is larger than the chi-square table value for a specified significance level.

Normality plot of residuals

Graphical tool used for comparing data set with normal distribution. From the nature of histogram one can easily identify whether it is normally distributed or not.

Results and Discussion

Box-Jenkins (1970) methodology was applied to model the rainfall data and it includes identification of the model, estimation of the model parameters and validation of the model (Hipel et al., 1977). The time series data should be stationary which means that it should have a constant mean, variance, and covariance which dependent only on time before fitting ARIMA models. The most used method to transform non-stationary data to stationary is differencing the data points, which replaces each value in the series by the difference between two consecutive values as t^{th} and $t-1^{th}$ periods for a first order differenced series.

Identification of the model

Stationarity was checked using unit root test (ADF test) and examining the autocorrelation function (ACE) and partial autocorrelation function (PACF) to identify the potential models. Null hypothesis for the ADF test was the presence of unit root indicating non-stationary and the alternate hypothesis as no unit root indicating a stationary time series. ADF test results of rainfall data in all the three stations were found to be in rejection

zone indicating stationarity and the order of integration is zero. Based on the significant value of the ADF test the order for integration for both non seasonal and seasonal component was detected and it is shown in Table 1.

Table 1. Order of integration based on unit root test result of rain fall data

Stations	ADF test P-value	Regular difference order	Seasonal difference order
Vellayani	-9.89	0	1
Kumarakom	-13.63	0	1
Pampadumpara	-13.28	0	1

Classical methods based on ACF and PACF were also performed to identify AR and MA components for both non-seasonal and seasonal parts. Fig.1, 2 and 3 shows

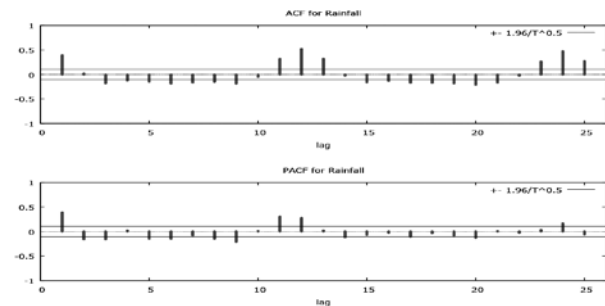


Fig. 1. ACF and PACF plot for rainfall at Vellayani, Thiruvananthapuram, Kerala

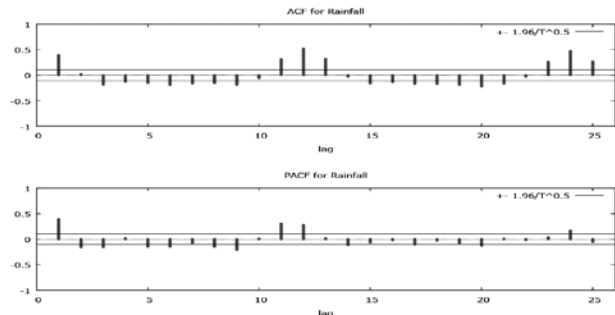


Fig. 2. ACF and PACF plot for rainfall at Kumarakom, Kottayam, Kerala

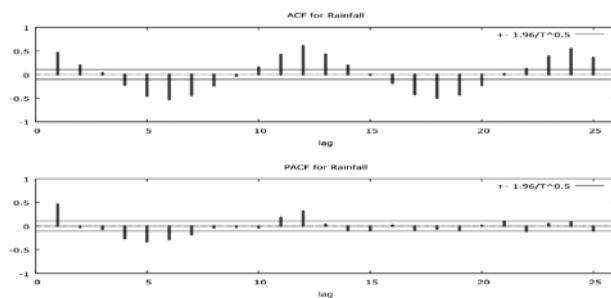


Fig. 3. ACF and PACF plot for rainfall at Pampadumpara, Idukki, Kerala

the correlogram corresponds to the rainfall data of the stations with lag length of 25 in X-axis and autocorrelation values in the Y-axis.

The seasonal autocorrelation relationship was observed and quite prominent from ACF and PACF and the gradual decay observed in the plot again indicate stationarity nature of data set. Based on the nature of the correlogram and the result of the unit root test we can choose a temporary model for rainfall and the model could be ARIMA (p, 0, q) (P, 1, Q).

Estimation of Parameters of the model

Even though the order of integration was identified the parameters and the best ARIMA model was identified by trial-and-error method based on the value of AIC, BIC and Hannan Quinn criteria. The different models estimated with different criteria using the open-sources of tware Gretl are shown in Table 2, 3 and 4.

For Vellayani, the model having p=1, d=0, q=0, P=0, D=1, Q=1 has lower values for AIC, BIC and Hanann

Table 2. ARIMA models for rainfall at Vellayani

ARIMA Model	Coefficient	P-value	AIC	BIC	Hannan Quinn	
(001)(111)	phi-1	0.07	0.33	4037.84	4060.74	4046.97
	theta-1	0.12	0.02**			
	theta-1	-0.89	3.39e-052***			
(003)(010)	theta-1	0.12	0.02**	4185.58	4208.49	4194.71
	theta-2	0.12	0.02**			
	theta-3	-0.03	0.53			
	phi-1	-0.44				
	phi-2	-0.70	0.003***0.00			
	phi-1	0.06	07***0.43			
(202)(111)	theta-1	0.56	2.49e-06***	4037.90	4072.25	4051.60
	theta-2	0.803460	1.44e-05***			
	theta-1	-0.893021	1.37e-05***			
(100)(011)	phi-1	0.14	0.009***7.1	4035.98	4055.07	4043.59
	theta-1	-0.85	5e-06***			

Table 3. ARIMA models for rainfall at Kumarakom

ARIMA Model	Coefficient	P-value	AIC	BIC	Hannan Quinn	
(000)(112)	theta-1	-1.01	0.0009***	4172.91	4191.99	4180.52
	theta-2	0.01	0.81			
	phi-1	0.07	0.19			
(100)(111)	phi-1	-0.02	0.77	4173.18	4196.08	4182.31
	theta-1	-0.99	0.003***			
	phi-1	-0.66	0.0004***			
	phi-1	-0.005	0.93			
(101)(111)	theta-1	0.74	7.75e-06***	4173.22	4199.95	4183.88
	theta-1	-1.00	5.66e-09***			
(100)(110)	phi-1	0.12	0.02**	4250.59	4269.68	4258.19
	phi-1	-0.55	1.20e-32***			
(000)(011)	theta-1	-1	2.14*10 ⁻¹²	4170.97	4186.23	4177.05

Table 4. ARIMA Models for Rainfall in Pampadumpara

ARIMA Model	Coefficient	P-value	AIC	BIC	Hannan Quinn	
(001)(112)	phi-1	0.708	0.0002***	3994.501	4021.221	4005.152
	theta-1	0.046	0.3788			
	theta-1	-1.66	5.76e-022***			
	theta-1	0.711	1.82e-06***			
(101)(012)	theta-2			3996.582	4023.302	4007.233
	phi-1	0.669	0.0084***			
	theta-1	-0.608	0.0239**			
	theta-1	-0.907	4.61e-048***			
(100)(110)	theta-2	0.029	0.6301	4088.251	4107.336	4095.859
	phi-1	0.080	0.1404			
	phi-2	-0.499	1.93e-024***			
	theta-1	0.88	1.46*10-144***			

Quinn criterion which revealed the best model for rainfall was ARIMA (1, 0, 0) (0, 1, 1)₁₂. The model having p=0, d=0, q=0, P=0, D=1, Q=1 has lower values for AIC, BIC and Hanann Quinn criterion for Kumarakom data which revealed that the best model was ARIMA (0, 0, 0) (0, 1, 1)₁₂. Pampadumpara model having p=0, d=0, q=0, P=0, D=1, Q=1 has lower AIC, BIC and Hanann Quinn criterion and so the best model for rainfall was ARIMA (0, 0, 0) (0, 1, 1)₁₂. All the coefficients of the estimated models were highly significant since p-values were less than 0.05. Fig. 4, 5 and 6 shows the plot for actual and fitted values, where there dlinere presents the actual values and blue line represent the fitted values

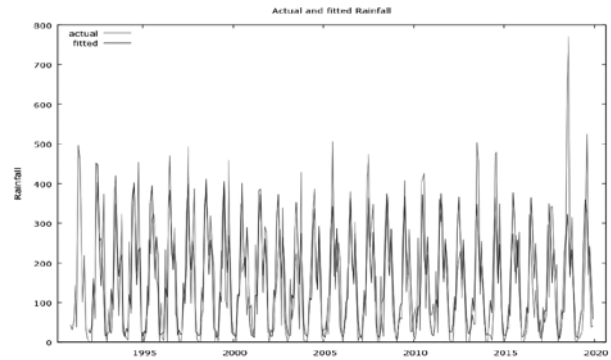


Fig. 6. Actual versus fitted plot for rainfall in Pampadumpara

and from the graph it is obvious that fitted and actual were closer.

Model validation

The best fitted model for rainfall in Vellayani was found to be ARIMA (1,0, 0) (0, 1,1)₁₂.

The functional form of the model is:

$$y_t - y_{t-12} = \phi_1 y_{t-1} - \phi_{12} y_{t-13} + \Theta_1 e_{t-12} + e_t$$

Let $y_t - y_{t-12} = Z_t$ then,

$$Z_t = \phi_1 Z_{t-1} + \Theta_1 e_{t-12} + e_t$$

Here $\phi_1 = 0.14$ and $\Theta_1 = -0.85$

$$Z_t = 0.14 Z_{t-1} - 0.85 e_{t-12} + e_t$$

The best fit model for rainfall in Kumarakom was ARIMA (0, 0, 0) (0, 1, 1)₁₂. The functional form of the model is:

$$y_t - y_{t-12} = \Theta_1 e_{t-12} + e_t$$

Let $y_t - y_{t-12} = Z_t$ then,

$$Z_t = \Theta_1 e_{t-12} + e_t$$

Here, $\Theta_1 = -1$

$$Z_t = -e_{t-12} + e_t$$

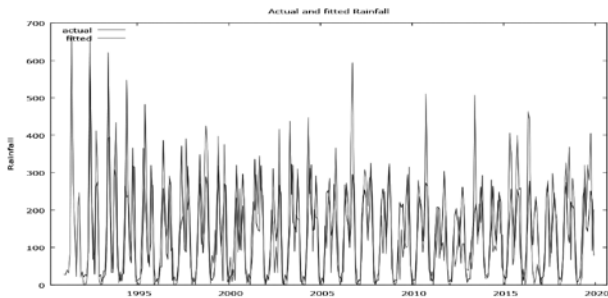


Fig. 4. Actual versus fitted plot for rainfall in Vellayani

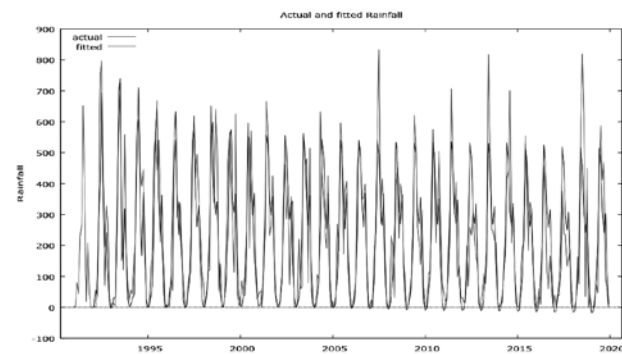


Fig. 5. Actual versus fitted plot for rainfall in Kumarakom

The best fit model for rainfall in Pampadumpara was ARIMA (0, 0, 0) (0, 1, 1)₁₂. The functional form of the model is:

$$y_t - y_{t-12} = \Theta_1 e_{t-12} + e_t$$

Let $y_t - y_{t-12} = Z_t$ then,

$$Z_t = \Theta_1 e_{t-12} + e_t$$

Here $\Theta_1 = 0.8$

$$Z_t = 0.88 e_{t-12} + e_t$$

Two methods are commonly used to test the adequacy of the selected model one method is by checking the autocorrelation of the residuals using Ljung-Box Q test (Box et al., 1995) and second is by checking the normality of the residuals. It has been found to measure the overall adequacy of the chosen model by examining a quantity Q known as Ljung-Box statistic (Yurekli et al., 2005; Sallehuddin et al., 2007), which is a function of autocorrelations of residuals and its approximate distribution was Chi-square. If Ljung-Box statistic value is found non-significant then residuals are uncorrelated and hence the model selected was good enough for the prediction. The estimated Ljung-box test statistic of rainfall at three stations are shown in Table 5, result indicated that the residuals are not correlated. Fig. 7 displays the normality plot of residuals for rainfall and it clearly shows that residuals are normally distributed.

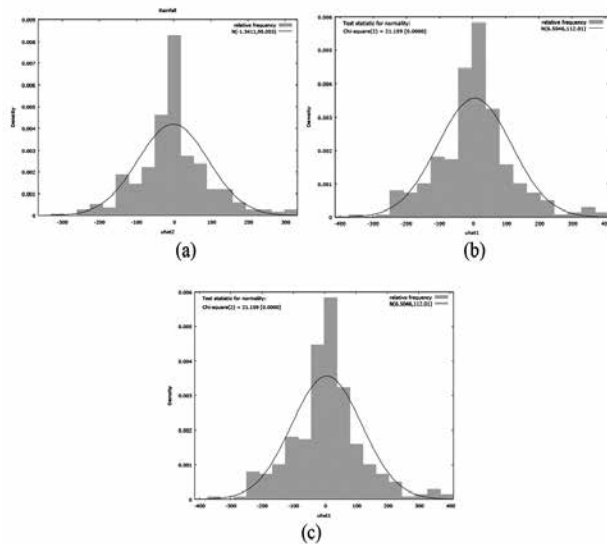


Fig. 7. Normality plot of residuals for rainfall at (a) Vellayani, (b) Kumarakom and (c) Pampadumpara

Tab. 5. Result of Ljung-Box test

Station	Ljung-box test statistics	p value
Vellayani	8.08	0.62
Kumarakom	7.57	0.757
Pampadumpara	9.74	0.55

Conclusions

The SARIMA model was fitted to monthly rainfall for all the regions Vellayani, Kumarakom, and Pampadumpara using the monthly data for the period from 1991 to 2019. The model parameters were obtained by using maximum likelihood method and the best model were selected using Akaike Information Criteria (AIC), Bayesian Information Criteria (BIC) and Hannan-quinn coefficient. ARIMA (1, 0, 0) × (0, 1, 1)₁₂ was found best fit for rainfall for Vellayani, ARIMA (0, 0, 0) × (0, 1, 1)₁₂ for Kumarakom and Pampadumpara. The adequacy of the check of the selected models confirmed that the selected models were free from autocorrelation and the residuals are normal.

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Productivity and profitability of taro (*Colocasia esculenta* (L.) Schott) under drip and furrow irrigation

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Abstract

Productivity and profitability of taro under drip irrigation and furrow irrigation was worked out based on the data collected from field experiments carried out at ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India, for three years (2016-17 to 2018-19). The experiment was conducted in RBD, with upland taro variety, Muktakeshi, during summer, with seven treatments which included five levels of drip irrigation, furrow irrigation and a rainfed crop and three replications. The mean data over a period of three years indicated that drip irrigation @ 100% CPE resulted in the highest cormel yield, gross and net income and B:C ratio in taro. In addition to saving of irrigation water (30%), drip irrigation resulted in 45% increase in cormel yield, 57% increase in net income and 20% increase in B:C ratio, compared to furrow irrigation.

Keywords: B:C ratio, Taro, Drip irrigation, Productivity, Profitability

Introduction

Taro (*Colocasia esculenta* (L.) Schott) is an herbaceous perennial root crop, widely cultivated in tropical and subtropical regions of the world. It is now grown in almost every area of the humid tropics. The corms and cormels are the major economic part of the crop. Depending on the cultivars and culture, the leaves, flowers, and petioles are also occasionally utilized as food (Fred and Makeati, 2001).

Taro is adapted to tropical lowlands with evenly distributed annual rainfall of 2000 mm, high temperatures of 20-35°C and shaded conditions. It grows best in well drained loamy soils but can be grown in a wide range of soils including sandy, clay and loamy soils with pH ranging from 5.5 to 6.5 (Onwueme, 1999). Two main production systems exist in taro cultivation, the flooded or low land taro production, where water is available

throughout, and the water level can be controlled and the dry land taro or upland taro, which is rain-fed and often has to be supplemented with irrigation to realise the expected yield. Taro is reported to be one of the least water efficient crops and upland varieties may be adapted to water limited conditions (Uyeda et al., 2011). Li Meiling et al., (2019) reported that sandy soil has greater potential to improve the water use efficiency (WUE) of taro under limited water availability conditions. The average productivity of taro is the highest in Asia (16.5 t ha⁻¹) and the lowest in Africa (4.3 t ha⁻¹) and the world average productivity is reported as 5.39 t ha⁻¹ (<http://www.fao.org/faostat>).

In India, taro is mainly cultivated in the states of Uttar Pradesh, Madhya Pradesh, Andhra Pradesh, Odisha, Telangana, and the Northeastern hilly areas. In other places, it is cultivated on a limited scale as intercrop or

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homestead crop but consumed as a routine vegetable or food crop by all sections of people. The crop is mostly cultivated with monsoon rains, quite often needs supplemental irrigation, using furrow system. Most of the varieties and land races are season insensitive and can be grown in any part of the year, provided sufficient soil moisture is assured. Being a moisture sensitive crop, taro responds well to irrigation and drip irrigation is established to be a successful practice in enhancing irrigation water use efficiency and water productivity in many crops. In the present study, a comparison is made between furrow irrigation and drip irrigation in upland taro cultivation in terms of productivity and profitability.

Materials and Methods

The data were collected from the field experiments carried out in taro, during the three consecutive summer seasons of 2016-2017, 2017-2018 and 2018-2019 at ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India. In all the seasons, the crop was planted in November and harvested in May. Based on the USDA taxonomic system, the soil was classified as sandy clay loam having 62% sand, 10% silt and 28% clay content. The soil of the experimental area was medium in available nitrogen (252 kg ha⁻¹), high in available phosphorus (121 kg ha⁻¹) and medium in available potassium (188 kg ha⁻¹).

During the first season, minimum and maximum temperature varied from 21.9 to 24.3°C and 31.5 to 33.9°C respectively with a total rainfall of 180 mm and relative humidity ranging between 70.1 to 80.9%. Second season received a rainfall of 352 mm, minimum and maximum temperature 21.5 to 24.9°C and 30.1 to 34.2°C respectively, relative humidity from 52 to 76.6% were recorded. Minimum and maximum temperature varied from 20.8 to 25.3°C and 31.2 to 34.4°C respectively, relative humidity from 68.1 to 83.4%, during the third season, receiving a rainfall of 251 mm.

The experiment was conducted in Randomized Block Design, with five levels of drip irrigation, and two controls, furrow irrigation and rainfed crop replicated thrice. The five levels of drip irrigation were 50% (I₁), 75% (I₂), 100% (I₃), 125% (I₄) and 150% (I₅) of cumulative pan evaporation (CPE), based on the daily evaporation data collected from a Class B open pan evaporimeter, placed near the site. Taro variety 'Muktakeshi' was used for the study. This variety was released during 2002 by the Regional Station of ICAR-Central Tuber Crops Research Institute, Bhubaneswar, Odisha. It is a clonal selection from Bhatpara, a collection from Cuttack, Odisha. The variety is resistant to *Phytophthora* leaf blight disease and is suitable for cultivation both in uplands as well as in lowlands. It is comparatively a short duration variety

having 6-7 months duration with an average yield of 17-20 t ha⁻¹ under good management conditions.

Small pits were taken in rows at a spacing of 60 cm and cormels of uniform size (25 to 30 g) were planted at a spacing of 45 cm. As per the package of practices recommended by ICAR-CTCRI, 80 kg N, 25 kg P and 100 kg K were applied in three split doses. One third dose of N and K, and full dose of P were applied two weeks after initiation of sprouting, remaining N and K at one month interval in equal splits. Agronomic practices were the same in all the seasons.

Drip system was laid out and drippers were placed so as to coincide with the spacing of the plants. Each plot had 36 plants with a net plot size of 16 plants. Irrigation was given through drip system, and the flow of water was controlled using a drip meter. Furrow irrigation was given twice a week @ 5 mm per day. For rainfed crop, only life-saving irrigation was given, whenever there was no rain continuously for a week. The crop was harvested after seven months, corm yield and cormel yield were recorded from different treatments from the net plot during the three growing seasons and based on the yield data from net plots, per hectare yield was estimated in t ha⁻¹. Economic indices viz., cost of cultivation, gross income, net income and benefit: cost ratio were worked out based on various inputs and labour costs at the end of three years. The data over the three years were pooled and analyzed statistically following Indian NARS Statistical computing portal (SSCNARS) by applying the technique of Analysis of Variance (ANOVA) for RBD and multiple comparison of treatment means was done by least significant difference.

Results and Discussion

Corm and cormel yield

Pooled analysis of data of three seasons showed significant variation among treatments for yield. Seasons did not impart significant effect on yield. The cormel yield and total yield varied significantly among different drip irrigation levels. There was no significant variation in corm yield among the treatments. The cormel yield increased from 13.18 to 21.08 t ha⁻¹ under drip irrigation levels from 50% to 100% CPE and thereafter declined. There was 45% increase in cormel yield under drip irrigation at 100% CPE, compared to furrow irrigation. Rainfed crop resulted in cormel yield of 3.47 t ha⁻¹. Corm yield was the highest with irrigation at 125% CPE and the total yield (corm + cormel) was the highest at 75% CPE. Cormel to corm ratio did not show any definite trend with increase in drip irrigation from 50% to 150% CPE, however, the value was the highest at ET_c 100% (Table 1).

Table 1. Cormel yield, corm yield and total yield of taro (t ha⁻¹) under different irrigation treatments (Pooled mean of three seasons)

Treatment	Cormel	Corm	Total	Cormel/ Corm ratio
T1	13.18	8.52	20.58	1.32
T2	17.71	13.12	34.39	1.35
T3	21.08	12.88	31.06	1.64
T4	18.8	13.92	29.49	1.42
T5	18.26	12.18	32	1.50
T6	14.47	10.39	24.86	1.39
T7	3.47	3.28	7.43	1.06
CD	6.563	NS	13.612	0.252

Corm yield was more under lower levels of irrigation, but cormel yield was more with higher levels of drip irrigation, though the values were not statistically different under different irrigation levels. More number of tillers produced under lower levels of irrigation might have resulted in more corm yield. It is also evident from the values of cormel to corm ratio, which was the highest for 100% CPE (1.64) but was comparable to 125% CPE and 150% CPE. Low land production systems and the upland production supplemented with irrigation is a must for realising good yield in taro. Irrigation would be beneficial for taro production in drier months as well as low rainfall areas (Sunitha et al., 2022). In field experiment in taro with different irrigation water levels of 50, 75 and 100% ET_c, ET_c at 50% resulted in the highest reduction in terms of vegetative growth, yield characteristics, yield and bio constituents compared to 75% of ET_c level and unstressed plant (100% of ET_c) (El Aal et al., 2019). In yet another study, *in-situ* moisture conservation methods influenced soil water availability and subsequent vegetative growth and yield of taro under upland conditions (Manyatsi et al., 2011). Increased cormel yield in taro (Mabhaudhi et al., 2013) and tuber yield in potato (Badr et al., 2012) is reported with increase in amount of water applied. In the present study also, the highest cormel yield was observed for irrigation at 100% CPE, beyond which the yield showed a declining trend. In potato, Camargo et al., (2015) found 80% of irrigation requirements showed statistically similar yields to 100 and 120% of irrigation requirements.

Cost of installation of drip irrigation unit

The cost of irrigation materials depends mainly on the distance of the field from the water source. Since taro is planted at a closer spacing of 60×45 cm, a greater number of drippers are required. The total cost of installation in one ha of area comes to be about ₹2.25 lakhs (Table 2) including accessories and installation charges. After considering the depreciation, maintenance

cost etc. during subsequent years, the cost of fertigation unit comes to about ₹60,250 per year.

Table 2. Cost of installation of drip irrigation (Area: 1 ha) (Fixed cost) (₹ ha⁻¹)

No.	Particulars	Cost of laterals, drippers etc.	Cost of pipes, valve, motor, filters etc.
1	Fixed cost (×10 ³)	1.50	0.75
2	Life year	6	20
3	Depreciation (×10 ³)	25.0	3.75
4	Interest (12%) (×10 ³)	18	9
5	Repair and maintenance (2%) (×10 ³)	3.0	1.5
	Total	46	14.25
	Grand total	60.25	

Cost of cultivation

The cost of cultivation of taro under different levels of drip irrigation was worked out and it ranged from ₹227400 to ₹292450. The variation was mainly due to the difference in irrigation water applied (Table 3). Under the rainfed conditions, the cost of cultivation was only ₹160200 ha⁻¹.

Table 3. Cormel yield and economics of taro cultivation under drip and furrow irrigation

Treatment	Cormel Yield (t ha ⁻¹)	Cost of cultivation (₹ ha ⁻¹)	*Gross income (₹ ha ⁻¹)	Net income (₹ ha ⁻¹)	B:C Ratio
T1	13.18	267450	659000	391550	2.46
T2	17.71	271450	885500	614050	3.26
T3	21.08	275950	1054000	778050	3.82
T4	18.8	279950	940000	660050	3.36
T5	18.26	292450	913000	620550	3.12
T6	14.47	227400	723500	496100	3.18
T7	3.47	160200	173500	13300	1.08
CD	6.563				0.48

*Price of taro@ ₹50000 per tonne

Gross income and Net income

The gross income ranged from ₹659000 to 1054000 under drip irrigation treatments and net income from ₹391550 to 778050 per ha. The gross and net income from rainfed crop was ₹1,73,500 and 13,300, respectively. The highest net income was obtained from T3, *i.e.*, irrigation at 100% CPE. The minimum was for T1, *i.e.*, drip irrigation at 50% CPE. Positive response from drip irrigation was evident upto irrigation at 100% CPE, from more yield, which consecutively resulted in more gross and net income. Drip irrigation resulted in

45.68% increase in cormel yield compared to furrow irrigation and hence it was more profitable, though the initial investment for installation of drip irrigation facility was incurred.

B:C Ratio

B:C ratio also followed a similar trend as in gross and net income. The ratio ranged from 2.46 to 3.82 under drip irrigation, whereas furrow irrigation and rainfed control resulted in B:C ratio of 3.18 and 1.08 respectively. Similar increase in yield, gross and net income, and B:C ratio under drip irrigation over flood irrigation due to increased water and nutrient use efficiencies in tuber crops, have been reported (Nedunchezhiyan, 2017; Sunitha et al., 2018).

Productivity, profitability and relative economic efficiency

The crop was of seven months duration and productivity in terms of cormel yield and profitability in terms of profit/day were worked out. Based on pooled means, the productivity per day was 1.4 times and profitability $\text{ha}^{-1} \text{day}^{-1}$ was 1.6 times higher under drip irrigation compared to furrow irrigation. Relative economic efficiency (which is a measure of increase in net income over control) was worked out to be 56.8% over furrow irrigation. Maximum productivity per day and profitability $\text{ha}^{-1} \text{day}^{-1}$ were recorded by drip irrigation at 100% CPE (Fig.1). In this experiment, taro yielded 45% more yield under T3, compared to furrow irrigation, which resulted in more gross and net income and B:C ratio, productivity and profitability, in addition to saving of almost 30% irrigation water.

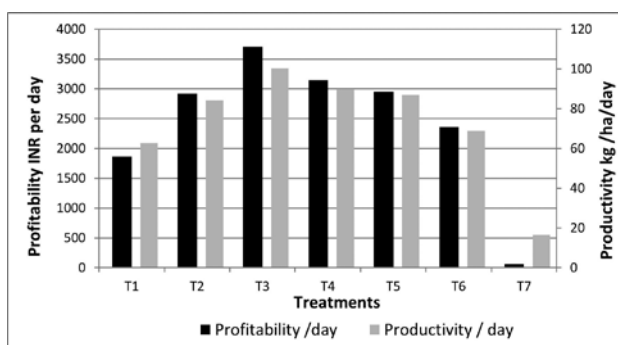


Fig. 1. Productivity and Profitability of taro cultivation under different irrigation regimes

Conclusion

The above findings clearly revealed that taro cultivation under drip irrigation was economical compared to that under furrow irrigation. The pooled mean of data

over three years indicated that drip irrigation @ 100% cumulative pan evaporation resulted in highest cormel yield, gross and net income, B:C ratio and profitability per ha per day in upland taro during summer months.

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Strategies for enhancing post-harvest quality and shelf life of tuber crops: Insights from physiological perspectives

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Abstract

This comprehensive review explores various strategies aimed at improving the quality and extending the shelf life of tubers such as potato, cassava, sweet potato and yams after they are harvested. It focuses on the physiological aspects involved in post-harvest storage. The review delves into the changes that occur within the tuber crops during storage, such as metabolic and biochemical transformations, shifts in respiration rates and ethylene production, and modifications in the composition and texture of cell walls. Additionally, the review addresses common physiological disorders that can arise during the storage of tuber crops, discussing their causes and the impact of storage conditions on their development. The review further provides insights into pre-harvest considerations, optimized harvesting techniques, post-harvest treatments for disease and pest control, and the optimization of storage conditions to maximize the shelf life of tuber crops. It emphasizes the significance of physiological markers and indicators in assessing tuber quality and their role in making informed decisions during the post-harvest phase. The review also explores advancements in post-harvest technologies, including modified atmosphere storage, cold storage, and innovative approaches for maintaining quality and inhibiting sprouting and discusses emerging trends in post-harvest physiology research, the challenges and opportunities for enhancing tuber crop quality, and potential areas for future investigation.

Keywords: Tuber crops, Post harvest management, shelf life, physiological properties, dormancy

Introduction

Background and significance

Tuber crops, including potatoes, sweet potatoes, yams, cassava, and aroids have a significant impact on global food security and livelihoods, acting as essential staple foods for millions of people worldwide. These crops provide vital nutrients and contribute to dietary diversity. Fresh tubers are highly perishable and prone to post-harvest losses, resulting in considerable economic ramifications and diminished food availability. Post-harvest losses

in perishable crops, encompassing fruits, vegetables, and tubers present formidable challenges to both food security and economic sustainability. In developing countries, post-harvest losses after harvest can reach as high as 40% for fruits, vegetables, and root crops (Atanda et al., 2011; Kiaya, 2014). Multiple factors contribute to these losses such as inefficient harvesting, packaging, and handling practices, as well as fluctuations in temperature and humidity, pathogenic infections, and damage caused by insects and rodents (Atanda et al., 2011; Kiaya, 2014). Several strategies can be implemented to mitigate

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these challenges. Enhancing harvesting, packaging, and handling practices is paramount to minimize mechanical damage and exposure to extreme conditions (Atanda et al., 2011; Kiaya, 2014). Furthermore, establishing appropriate storage conditions, including maintaining optimal temperature and humidity levels, can effectively prevent spoilage (Atanda et al., 2011; Kiaya, 2014). Effective pest control methods are also crucial in safeguarding crops against pests and pathogens (Atanda et al., 2011). Additionally, the utilization of improved varieties and rootstocks plays a pivotal role in reducing post-harvest losses by bolstering resistance to pests, diseases, and environmental stresses while simultaneously prolonging the shelf life of tuber crops (Atanda et al., 2011; Kiaya, 2014; Kader and Rolle, 2004). Proper post-harvest management practices assume vital significance in ensuring the quality, safety, and market value of horticultural produce, particularly for root, tuber, and bulb crops (Kader and Rolle, 2004).

The post-harvest phase is crucial in determining tuber crop quality and shelf life. This phase encompasses physiological and biochemical changes impacting sensory attributes, nutritional composition, and market value. These changes include weight loss, sprouting, enzymatic browning, softening, and the accumulation of toxic compounds. Researchers and stakeholders have been actively involved in developing strategies to improve post-harvest quality and prolong the shelf life of tuber crops. Tuber crops possess underground storage organs that store significant amounts of starch, minerals, vitamins, and other valuable components. However, the inherent physiological characteristics render them susceptible to rapid deterioration after harvest. Post-harvest losses in tuber crops can be attributed to enzymatic reactions, microbial growth, mechanical damage, and unfavourable storage conditions. These factors interact and accelerate the degradation processes, leading to a decline in quality and nutritional value.

Enhancing post-harvest quality and extending the shelf life of tuber crops hold immense importance for multiple reasons. Firstly, minimizing post-harvest losses contributes to global food security by ensuring a consistent supply of nutritious tuber crops throughout the year, particularly in regions where they serve as staple foods and provide primary calorie sources. Secondly, improving post-harvest characteristics reduces economic losses for farmers, traders, and other stakeholders involved in the supply chain. By extending the shelf life, farmers gain access to distant markets and can obtain higher prices for their produce. Lastly, enhancing the post-harvest quality of tuber crops aligns with sustainable development goals by reducing food waste and promoting efficient resource utilization.

In recent years, considerable research efforts have been devoted to understanding the physiological processes

involved in the post-harvest deterioration of tuber crops. These studies have contributed valuable insights into the factors that influence tuber quality and shelf life, leading to the development of innovative strategies to mitigate post-harvest losses. This review aims to provide a comprehensive understanding of the physiological aspects related to enhancing post-harvest quality in tuber crops while discussing promising strategies for preserving their freshness, nutritional value, and marketability. The objectives of this review are multifaceted. Firstly, it seeks to offer a comprehensive overview of the physiological processes that impact the post-harvest quality and shelf life of tuber crops. Researchers and stakeholders can identify critical intervention points and develop targeted strategies for preserving quality by gaining insights into the underlying mechanisms. Secondly, the review highlights recent advancements in post-harvest technologies, encompassing innovative storage techniques, packaging materials, and treatments that have demonstrated potential in enhancing post-harvest attributes. By examining scientific evidence and practical applications, this review aims to provide valuable insights for researchers, policymakers, and industry professionals engaged in the production, storage, and distribution of tuber crops.

Physiological changes during post-harvest storage of tuber crops

Tuber crops, including potatoes, sweet potatoes, taro, tannia, elephant foot yam, and yams, are globally recognized for their significant contribution to food security and nutrition. However, once harvested, the tubers and rhizomes undergo various physiological and biochemical changes that can impact their quality, nutritional composition, and shelf life. A comprehensive understanding of the metabolic and biochemical transformations occurring during post-harvest storage is essential for implementing adequate storage and preservation strategies. These transformations involve intricate interactions between enzymes, substrates, and environmental factors, resulting in alterations in carbohydrate, lipid, and protein metabolism, as well as the presence of phytochemicals. The primary objective of this review is to delve into the metabolic and biochemical transformations that occur during the post-harvest storage of tuber crops and explore their implications for the quality and preservation of these invaluable food resources.

Metabolic and biochemical transformations

Metabolic and biochemical transformations occur during the post-harvest storage of tuber crops, influencing their quality and shelf life (Uritani, 1999). These transformations encompass a range of metabolic processes and biochemical reactions that impact the tubers' composition, texture, and overall state. Notably,

carbohydrate metabolism assumes a significant role in tuber crops, as the enzymatic breakdown of starch, the primary carbohydrate reserve, results in the formation of soluble sugars, which contribute to sweetness, flavour, and texture (Ngadze et al., 2018). Lipid metabolism also experiences changes during storage, with the potential for lipid degradation and oxidation, leading to undesirable flavors, rancidity, and alterations in nutritional profile (Kader, 2002). Furthermore, protein degradation occurs, leading to the breakdown of proteins into amino acids, thereby influencing texture, nutritional value, and sensory attributes (Ngadze et al., 2018). Enzymatic activities, including those of amylase, glucanase, and other enzymes, play a role in starch conversion, protein breakdown, and lipid degradation, thereby impacting the overall quality of tubers. Moreover, the levels of phytochemicals, such as phenolics and antioxidants, can change storage, influenced by factors such as temperature, light exposure, and oxygen availability. Managing storage conditions, encompassing temperature, humidity, and handling practices, is critical in controlling these metabolic and biochemical transformations and preserving tuber crops' quality and nutritional value.

Changes in respiration rates and ethylene production

Apart from metabolic and biochemical changes, ethylene and oxidative damages play significant roles in the post-harvest storage of tuber crops. Ethylene, a plant hormone, can be induced during post-harvest stages and affects various physiological processes, including cell wall changes and tissue softening (Yahia and Carrillo-Lopez, 2018; Dong et al., 2020). Ethylene exposure can lead to alterations in cell wall components, contributing to texture changes in tubers (Dong et al., 2020; Reilly et al., 2007). Furthermore, oxidative damages caused by increased susceptibility to pathogens (Yahia and Carrillo-Lopez, 2018; Martinez-Romero et al., 2007). Proper management of storage conditions, including temperature, humidity, and handling practices, assumes a crucial role in controlling these transformations, ethylene effects, and oxidative damages, thereby preserving the quality and nutritional value of tuber crops (Ravi and Aked, 1996; Uritani, 1999; Yahia and Carrillo-Lopez, 2018).

Changes in respiration rates and ethylene production are observed in tuber crops during post-harvest storage (Hirose et al., 1984). Respiration, a metabolic process involving carbohydrate breakdown and energy release, can increase in tuber crops due to factors like injury or biochemical changes (Hirose et al., 1984; Hajirezaei et al., 2003). Increased respiration rates can deplete stored nutrients and contribute to metabolic activity and tuber deterioration (Hajirezaei et al., 2003). Ethylene, a natural plant hormone, regulates physiological processes, including ripening and senescence. Tubers produce

ethylene during storage, impacting their quality and shelf life (Martinez-Romero et al., 2007). Ethylene accelerates ripening, tissue softening, and the formation of aroma and flavor compounds in tubers (Martinez-Romero et al., 2007; Yahia and Carrillo-Lopez, 2018). Reactive oxygen species (ROS) cause oxidative damage during tuber crop storage (Yahia and Carrillo-Lopez, 2018). ROS induces oxidative stress, leading to cellular damage, membrane deterioration, and decreased tuber quality (Yahia and Carrillo-Lopez, 2018). Antioxidant systems in tuber tissues mitigate ROS and maintain tuber quality (Mu et al., 2021). Various strategies can be employed to minimize the adverse effects of ethylene and oxidative damage during post-harvest storage. These include using ethylene inhibitors or scavengers to control ethylene levels and applying antioxidants or modified atmosphere packaging to reduce oxidative stress (Yahia and Carrillo-Lopez, 2018).

Dormancy regulation in tuber crops

Definition and types of dormancy

Dormancy is a critical physiological process in tubers that enables them to endure unfavourable conditions and maintain long-term viability. It involves suspended growth and metabolic activity until suitable conditions for sprouting and growth are present.

Two primary types of dormancies are observed in tubers:

Endodormancy: Endodormancy is an internal form of dormancy regulated by physiological factors within the tuber itself. During this period, tuber growth and metabolic processes are inhibited, and the tuber becomes unresponsive to external triggers for sprouting. Hormones such as abscisic acid (ABA) and ethylene suppress tuber sprouting and maintain dormancy (Gong et al., 2021; Mani et al., 2014).

Eco-dormancy: Eco-dormancy, also known as exodormancy, is influenced by external environmental factors. It occurs when external conditions, such as temperature, moisture, or photoperiod, are unfavourable for tuber growth and sprouting. Eco-dormancy prevents tubers from sprouting under unfavourable conditions, allowing them to conserve resources until more favourable conditions arise (Suttle, 2007).

Physiological and molecular mechanisms underlying tuber dormancy

The physiological age and genotype of tubers influence tuber dormancy. It initiates during tuberization and is determined by genetic factors, environmental conditions, and tuber age (Haider et al., 2021). Gaining insights into the molecular mechanisms that govern dormancy and sprouting is vital for devising strategies to manipulate dormancy in tuber crops.

The regulation of potato tuber dormancy and sprouting is a multifaceted process involving genetic, physiological, and environmental factors. Phytohormones play a pivotal role in controlling various stages of tuber development, including tuberization, initiation, growth, dormancy, and sprouting. Among these hormones, abscisic acid (ABA) and ethylene are crucial for regulating tuber dormancy and suppressing sprouting (Gong et al., 2021; Mani et al., 2014; Aksenova et al., 2013; Sonnewald and Sonnewald, 2014). These hormones act as inhibitors of sprouting, maintaining tuber dormancy during storage or unfavourable conditions. Although the exact mechanisms are still being investigated, molecular changes occur within the tuber during dormancy (Sonnewald and Sonnewald, 2014). The transition from dormancy to sprouting involves gene expression and hormonal metabolism, activating specific genes and biochemical processes (Agrimonti and Marmiroli, 2008). Additionally, non-structural sugar metabolism has been found to regulate tuber dormancy in certain yam species. Furthermore, environmental factors such as temperature, light, and humidity impact tuber dormancy and sprouting (Gong et al., 2021).

Factors influencing dormancy release and sprouting

Dormancy and sprouting in tuber and storage root crops are complex processes influenced by various factors. Phytohormones, including abscisic acid (ABA) and ethylene, play a crucial role in regulating dormancy and suppressing sprouting. Additionally, factors such as genetic manipulation, environmental conditions, sugar metabolism, and chemical treatments contribute to the control of dormancy and sprouting. Understanding these factors is essential for developing effective strategies to optimize storage conditions and manage the dormancy of tuber crops, ensuring their quality and viability.

Several factors have been identified as significant contributors:

Phytohormones: ABA and ethylene are involved in dormancy regulation and sprouting control in tuber crops.

Methyl jasmonate: Methyl jasmonate influences sprouting incidence in stored sweet potatoes and helps preserve overall quality (Véras et al., 2021).

Sugars: Sugar content in tubers affects dormancy, sprouting, and growth. Changes in sugar metabolism contribute to the transition from dormancy to sprouting.

Genetic factors: Genetic factors have a significant impact on tuber dormancy. Understanding the physiological and molecular basis of dormancy in tubers, such as yams, provides insights for genetic manipulation to control dormancy and sprouting.

Environmental factors: Temperature and photoperiod influence dormancy release and sprouting in tubers

(Cheema, 2010). Favourable conditions trigger sprouting, while unfavourable conditions can prolong dormancy.

Chemical treatments: Triadimefon and ethylene inhibitors are among the chemical treatments explored to inhibit sprouting and maintain tuber quality during storage (Lima et al., 2021).

Understanding these factors and their interactions is crucial for developing strategies to control dormancy release and optimize storage conditions in tuber and storage root crops.

Physiological Disorders in Stored Tuber Crops

Common physiological disorders and their causes

Physiological disorders in stored tuber crops are common occurrences that can significantly impact their quality and market value (Véras et al., 2021). Factors such as temperature, humidity, tuber age, and improper storage conditions contribute to the development of these disorders. One prevalent physiological disorder is sprouting, where shoots emerge from tubers during storage. Excessive sprouting leads to weight loss, firmness loss, and reduced tuber marketability (Véras et al., 2021). Physiological weight loss is another common disorder caused by the natural metabolic processes of tubers during storage. Respiration and transpiration of stored tubers contribute to weight loss, impacting overall tuber weight and quality (Lima et al., 2019). Environmental factors such as temperature and humidity can exacerbate weight loss in tubers. Internal discoloration or necrosis is a manifestation of physiological disorders in tuber crops. The accumulation of reducing sugars triggers Maillard reactions (Zhu et al., 2014), resulting in browning or darkening of tuber tissues. Improper storage conditions, high temperatures, and tuber injury contribute to internal discoloration and necrosis. In the case of cassava, post-harvest deterioration is a significant concern (Saravanan et al., 2015; Saravanan et al., 2016). Enzymatic and biochemical changes after harvest lead to quality degradation, loss of nutritional value, enzymatic browning, cyanogenic glucoside degradation, microbial spoilage, and textural changes. Chilling injury is another common physiological disorder observed in tuber crops stored at low temperatures. It causes tubers to become soft, develop surface pitting, and experience tissue breakdown (Cheema, 2010). Exposure to temperatures below the optimal range results in chilling injury and significant post-harvest losses.

Impact of storage conditions on disorder development

The impact of storage conditions on disorder development in tuber crops, including cassava, has been extensively studied and documented in the literature (Yan et al., 2016; Lalel et al., 2003; Bartz et al., 2009;

Mwitondi et al., 2021). Factors such as temperature, humidity, and ventilation are crucial in determining the extent of disorder development during storage. High temperatures during storage have been associated with an increased risk of physiological disorders in tuber crops. For instance, temperatures above 30°C have been found to promote the development of disorders such as vascular discoloration and internal necrosis in cassava (Yan et al., 2016). Curing is a postharvest treatment that can help to extend the storage life of root, tuber. This process plays a significant role in extending the shelf life of crops by promoting wound healing, strengthening the outer layers, and enhancing their overall quality (More et al., 2019). During curing, factors like temperature and humidity are carefully controlled to create an optimal environment for the crops. This promotes the sealing of wounds, reduces the risk of rot and spoilage, and helps retain moisture content, preventing excessive dehydration. Conversely, storing tubers at low temperatures can induce chilling injury, characterized by symptoms such as tissue softening, discoloration, and increased susceptibility to decay (Lalel et al., 2003). Humidity levels also influence disorder development in stored tubers. Excessive humidity can lead to increased water loss, promoting desiccation and shrivelling of tubers. Conversely, high moisture levels create a favourable environment for the growth of microorganisms, increasing the risk of rot and decay (Bartz et al., 2009).

Ventilation is another critical factor in tuber storage. Insufficient ventilation can result in the buildup of ethylene, carbon dioxide, and other metabolically produced gases, leading to accelerated deterioration and the development of disorders such as sprouting and internal browning (Mwitondi et al., 2021). By implementing appropriate storage conditions, including maintaining optimal temperature and humidity levels and providing adequate ventilation, the incidence, and severity of physiological disorders in stored tubers, including sweet potato and yams can be minimized. This

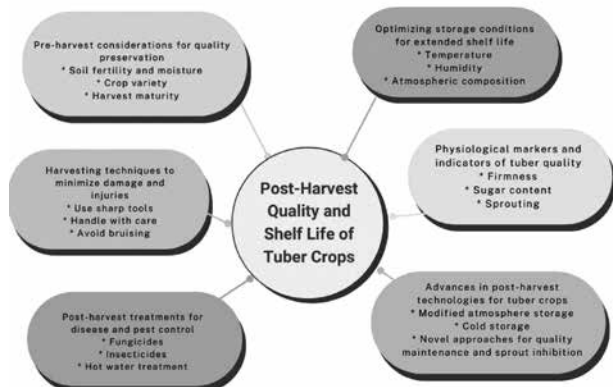


Fig. 1. Schematic diagram of physiological strategies for post-harvest quality and shelf-life of tuber crops

preservation of quality and nutritional value is essential in ensuring the marketability and usability of stored tuber crops.

Physiological approaches to mitigate disorders

Various physiological approaches can be utilized to mitigate physiological disorders in stored tubers and preserve their quality. These approaches encompass a range of techniques and treatments that specifically address underlying physiological processes and factors associated with disorder development. Some commonly employed physiological approaches are discussed below.

Temperature management: Proper temperature control is crucial for minimizing disorder development. Adjusting storage temperatures within optimal ranges for specific tuber crops can help reduce the incidence of disorders such as vascular discoloration, internal necrosis, and chilling injury (Kays, 1997). Specific temperature and humidity conditions for the curing of tubers can vary depending on the type of tuber and specific environmental factors. However, in general, optimal curing conditions for tropical tubers often involve temperatures ranging from 25°C to 35°C (77°F to 95°F) and relative humidity levels between 85% and 95%. For instance, sweet potatoes are commonly cured at temperatures around 30°C (86°F) with relative humidity maintained at approximately 90%. Cassava tubers, on the other hand, may require slightly higher temperatures, ranging from 32°C to 35°C (89.6°F to 95°F), while maintaining a relative humidity of 85% to 90% (Ravi and Aked, 1996; FAO, 1986)

Modified atmosphere storage (MAS): Creating a controlled atmosphere within storage facilities can help mitigate disorders. By regulating oxygen, carbon dioxide, and ethylene levels, MAS can effectively slow down physiological processes and delay the onset of disorders such as sprouting, decay, and internal browning (Valero et al., 2004).

Controlled humidity: Maintaining optimal humidity levels during storage is essential. Controlling humidity helps prevent excessive moisture loss, which can lead to tuber shrinkage, desiccation, and skin cracking. On the other hand, it also prevents excessive moisture buildup, which can contribute to rot and fungal growth (GraBmann et al., 2015).

Preharvest and post-harvest treatments: Various preharvest and post-harvest treatments can be applied to tubers to enhance their resistance to disorders. These treatments may include the application of protective coatings, antioxidants, fungicides, and growth regulators, which can help reduce oxidative stress, delay senescence, and inhibit microbial growth (Nguyen et al., 2021).

Hormonal regulation: Hormones play a significant role in tuber physiology and can be manipulated to mitigate disorders. For instance, applying plant growth regulators,

such as ethylene inhibitors, can delay sprouting and senescence processes, reducing the risk of sprouting-related disorders.

Implementing these physiological approaches in tuber storage practices can significantly reduce the occurrence and severity of physiological disorders, improve the shelf life, and maintain the overall quality of stored tubers. These strategies are essential for ensuring the marketability and usability of tuber crops in various agricultural and commercial contexts.

Strategies for post-harvest management of tuber crops

Pre-harvest considerations for quality preservation

Pre-harvest considerations are essential for preserving the quality of tuber crops. Various factors and techniques can be employed to improve post-harvest outcomes. Research shows that pre-harvest pruning can reduce the occurrence of rotten roots in sweet potatoes during storage (Tomlins et al., 2002). The application of foliar phosphonates has been found to suppress tuber infections of potato late blight (Mayton et al., 2008). When applied as a pre-harvest foliar treatment, Glyphosate shows the potential for suppressing sprout growth in stored potato tubers (Paul et al., 2014). Pre-harvest curing of sweet potato roots under tropical conditions can enhance skin adhesion, chemical composition, and shelf life (Parmar et al., 2017). Effective biocontrol treatments before harvest can reduce aflatoxin accumulation during drying (Kinyungu, 2019). Additionally, considering pre-harvest practices and storage conditions is crucial for maintaining quality and preventing losses in nectarines and potatoes during storage (Foukaraki et al., 2014). These pre-harvest considerations contribute to preserving tuber crop quality and storage outcomes.

Implementing appropriate strategies before harvest can help minimize the risk of physiological disorders and maximize the shelf life of tubers. Here are some critical strategies for pre-harvest quality preservation:

- **Optimal Harvest Time:** Harvesting tubers at the right stage of maturity is essential to maintain their quality during storage. Delaying harvest beyond the optimal stage can increase the risk of physiological disorders and reduce the storage life of tubers.
- **Proper Field Management:** Good field management practices, such as appropriate irrigation, fertilization, and pest control, are essential to promote healthy tuber growth and minimize the occurrence of diseases and pests that can impact post-harvest quality.
- **Integrated Pest Management (IPM):** Implementing IPM strategies helps control pests and diseases

sustainably. This approach combines various pest management techniques, including cultural practices, biological control, and judicious use of pesticides, to minimize chemical inputs while effectively managing pests and diseases.

- Tubers that are in good health generally exhibit an extended duration of storage as opposed to those that are damaged.
- **Disease and Pest Monitoring:** Monitoring tuber crops for diseases and pests allows for early detection and timely intervention. This can involve scouting the fields, inspecting plants for signs of diseases or pests, and taking appropriate measures, such as applying targeted treatments or removing infected plants to prevent the spread of pathogens or pests.
- **Proper Handling and Storage Practices:** Adequate care should be taken during harvesting, handling, and storage to prevent physical damage, bruising, and contamination of tubers. Gentle handling, using appropriate tools, and providing suitable storage conditions, such as optimal temperature and humidity levels, help maintain the quality and extend the shelf life of tubers.

Harvesting techniques to minimize damage and injuries

Harvesting techniques are crucial in minimizing damage and injuries to tuber crops. Research has shown that the choice of harvesting technique significantly affects tuber damage. Using new technology and avoiding mechanical injuries are essential in reducing tuber damage (Peters, 1996). In the case of potato tubers, mechanical injury during harvesting can be a significant concern, and factors such as the height of the drop and careful loading practices can impact the extent of injury (Zahara et al., 1961). For cassava roots, water loss from wounds caused during harvesting can lead to vascular discoloration and decreased quality (Marriott et al., 1978; Saravanan et al., 2015; Saravanan et al., 2016). Pre-harvest curing and preventing cuts, breaks, and skinning injuries can contribute to maintaining sweet potatoes' quality and shelf life (Tomlins et al., 2002). Similarly, mechanical damage during post-harvest handling of fruits and tubers is influenced by factors such as soil humidity and harvesting conditions (Martinez-Romero et al., 2004). Understanding and implementing proper harvesting techniques are essential for minimizing injuries and preserving the quality of tuber crops (Parmar et al., 2017; Ravi and Aked, 1996; Parmar et al., 2017).

Post-harvest treatments for disease and pest control

Post-harvest treatments for disease and pest control are crucial in preserving the quality of tuber crops. Storage roots of sweet potatoes are prone to various forms of

post-harvest losses, including sprouting, diseases, and pests (Ray et al., 2010). Effective post-harvest handling and storage methods significantly minimize these losses (Ray, 2015). The occurrence of post-harvest spoilage in sweet potatoes can be attributed to factors such as diseases, pests, and storage conditions. Among the pests, the sweet potato weevil is a significant concern (Ray and Ravi, 2005). Similarly, yam tubers are susceptible to post-harvest diseases and pests, emphasizing the need for proper handling, storage, and control measures (Okigbo, 2004). The application of foliar phosphite has shown promising results in reducing disease symptoms in post-harvest potato tubers (Lobato et al., 2011). Implementing appropriate storage methods and considering resistance traits in yam genotypes can help control post-harvest microbial rot (Nyadanu et al., 2014). It is vital to integrate genotype selection, storage methods, and biological control measures to minimize post-harvest losses caused by pests and diseases (Kiaya, 2014).

Optimizing storage conditions for extended shelf life

Optimizing storage conditions is crucial for extending the shelf life of tuber crops. Various factors, including curing treatments, storage temperature, humidity control, ventilation, light exposure, disease and pest control, and storage duration, play significant roles in ensuring the quality and longevity of tubers during storage. Curing treatments and storage temperature significantly influence the quality of Chinese yams during storage (Lee and Park, 2013). Proper control of water temperature and contact time is crucial in hot water treatment to inhibit the sprouting and spoilage of cured sweet potatoes without compromising their shelf life (Sheibani et al., 2014). Storage temperature plays a vital role in determining sweet potato tubers' storage stability and quality (Krochmal-Marczak et al., 2020). Changes in storage conditions or treatment can affect Chinese yam tubers' nutrient composition and sensory qualities (Zhang et al., 2014). Optimizing storage procedures, including humidity control, is necessary for the successful storage and sprouting prevention of yam micro tubers (Ovono et al., 2010). Standardization and refinement of storage procedures are essential for conserving and preserving tuber crops (Benson et al., 2011). Maintaining appropriate humidity levels prevents excessive moisture, which can lead to rot, fungal growth, and tuber dehydration. Optimal ventilation and airflow in storage facilities help regulate temperature and humidity, preventing the accumulation of ethylene, carbon dioxide, and moisture that can accelerate tuber deterioration and storage disorders. Tubers should be stored in darkness or under low-light conditions to avoid greening caused by chlorophyll accumulation and synthesize toxic compounds that can affect tuber quality and shelf life. Implementing effective disease and pest

control measures during storage is crucial to minimize post-harvest losses. Using appropriate fungicides, insecticides, or biocontrol agents helps prevent the spread of diseases and infestation by pests. Different tuber crops have specific storage durations that optimize their shelf life. Sweet potatoes, for example, have a relatively shorter shelf life and should be consumed within a few months, while certain potato varieties can be stored for several months under appropriate conditions. By carefully managing these factors, tubers can be stored for extended periods while maintaining quality and minimizing post-harvest losses.

Physiological markers and indicators of tuber quality

Non-destructive techniques play a crucial role in the quality assessment of root and tuber crops. Spectroscopic techniques, such as near-infrared reflectance spectroscopy and hyperspectral imaging, offer rapid and non-destructive evaluation of the quality of staple foods (Su et al., 2017). These techniques enable screening cassava storage roots for provitamin A carotenoids and assessing flesh color in sweet potatoes (Su et al., 2017; Sanchez et al., 2020). Other non-destructive methods, including X-ray imaging, laser light backscattering imaging, infrared thermal imaging, and ultrasonic technology, have also been applied for the quality evaluation of agricultural produce (Kotwaliwale et al., 2014; Chen and Sun, 1991; Farokhzad et al., 2020; Mizrach, 2008). X-ray imaging provides insights into internal quality, while laser light backscattering imaging and infrared thermal imaging offer non-destructive identification of fungal infections and quality assessment of foods, respectively (Kotwaliwale et al., 2014; Farokhzad et al., 2020; Sanchez et al., 2020). Ultrasonic technology enables fast and reliable evaluation of fresh fruit and vegetables during pre- and post-harvest processes (Mizrach, 2008). These techniques contribute to the detection of internal damage, identification of fungal infections, evaluation of texture, colour, and chemical composition, and overall quality assessment of tuber crops without causing physical harm to the samples (Farokhzad et al., 2020). They provide valuable tools for ensuring food safety, reducing post-harvest losses, and enhancing quality assurance in the storage and distribution of tuber crops (Sinha et al., 2017).

Role of physiological indicators in post-harvest decision making

Physiological indicators play a significant role in post-harvest decision-making. They provide valuable information about the quality and condition of agricultural produce, aiding in determining storage conditions, shelf life, and post-harvest treatments. Common indicators of post-harvest quality in fruits and vegetables include factors such as visual appearance, firmness, colour, aroma, taste, nutritional content, presence of decay or

physical damage, and overall shelf-life (Barbosa-Cánovas et al., 2003). In the context of post-harvest losses in India's fruit and vegetable supply chain, a study identified thirty indicators to evaluate critical causal factors and guide policy decisions (Gardas et al., 2018). In the case of post-harvest processing of Norwegian farmed salmon, measurable indicators were specified to reduce food loss (Abualtaher and Bar, 2020). The choice of post-harvest technology and storage decisions is influenced by multi-criteria methodologies considering different indicators, such as efficiency, cost-effectiveness, and environmental impact (Lenin et al., 2014). For the smallholder farmers' post-harvest decisions, including storage and processing, the risk and time preferences are influencing criteria (Ruhinduka et al., 2020). Understanding the key factors leading to post-harvest losses and waste involves analysing performance criteria and their indicators. For specific crops like potatoes and sugar beets, storage decisions are crucial for minimizing post-harvest losses. Assessing the quality before storage and considering long-term storage effects are essential for making informed decisions (Bachmann and Earles, 2000). Economic and logistics indicators also play a role in assessing post-harvest loss reduction strategies (Gunasekera et al., 2017). The use of physiological indicators in post-harvest decision-making ranges from assessing ripeness and quality to considering efficiency, risk preferences, and environmental impact. By incorporating these indicators, stakeholders can make informed decisions to minimize post-harvest losses and optimize the storage and processing of agricultural produce.

Advances in post-harvest technologies for tuber crops

Modified atmosphere storage and controlled atmosphere storage

Modified atmosphere storage (MAS) and controlled atmosphere storage (CAS) are advanced post-harvest technologies that play a crucial role in extending the shelf life of vegetables, fruits, and tubers. The MAS involves modifying the composition of the surrounding atmosphere, typically by reducing oxygen levels and increasing carbon dioxide levels, to create an optimal storage environment (Rao, 2015). This technique effectively inhibits respiration, slows down metabolic processes, and reduces microbial growth, thereby delaying spoilage. On the other hand, CAS takes the concept of modified atmosphere storage further by precisely controlling the gas composition, temperature, and humidity, creating an ideal storage condition for tuber crops (Aharoni et al., 2007). By customizing and optimizing storage conditions based on specific crop requirements, CAS has shown remarkable success in minimizing weight loss, retarding sprouting, reducing physiological disorders, and preserving overall quality

attributes of tuber crops such as potatoes and sweet potatoes (Rao, 2015; Aharoni et al., 2007). These technologies hold immense potential in improving post-harvest management practices and ensuring extended storage life for tuber crops, thus benefiting producers and consumers.

Cold storage and refrigeration technologies

Cold storage and refrigeration are essential for preserving the quality and prolonging the shelf life of tubers. Lower temperatures, such as 4°C, have decreased polyphenol oxidase (PPO) activity in potatoes and sweet potatoes, reducing browning and maintaining overall quality during storage (Sun et al., 2011). Ultrasound treatment has been shown to inhibit browning and improve the antioxidant capacity of fresh-cut sweet potatoes throughout the refrigeration period (Pan et al., 2020). In the case of sweet potato tuberous roots, a combination of low-temperature conditioning and cold storage promotes rapid sweetening while preserving quality (Li et al., 2018). Jerusalem artichoke tubers can benefit from refrigerated storage at zero degrees Celsius with a relative humidity of 90% (El-Awady and Ghoneem, 2011). These studies collectively emphasize the importance of cold storage and refrigeration techniques in preserving the quality and extending the storage life of tubers.

Novel approaches for quality maintenance and sprout inhibition

Advancements in post-harvest technologies for tuber crops have focused on novel approaches for sprout inhibition and quality maintenance. One such approach is the application of essential oils as a natural and alternate method for inhibiting and inducing the sprouting of potato tubers (Shukla et al., 2019). This eco-friendly method offers an alternative to using harmful chemicals and maintaining expensive cold storage conditions. Additionally, nonthermal treatments have shown promise in enhancing the shelf stability of fresh-cut potatoes, with novel nonthermal techniques demonstrating inhibitory effects on potato tuber sprouting (Rashid et al., 2021). Evaluating ecologically acceptable sprout suppressants has also been explored to enhance dormancy and potato storability, providing a wide range of options to prevent sprouting and maintain tuber quality (Gumbo et al., 2021). Furthermore, using microcapsules containing methyl jasmonate has shown preserving effects on post-harvest potato tubers, inhibiting sprouting and maintaining quality attributes. These advancements offer valuable insights into improving tuber crop sprout inhibition and quality maintenance.

Future Directions and Challenges

Emerging trends in post-harvest physiology research

The field of post-harvest physiology research is continuously evolving, and several emerging trends offer

exciting prospects for the future. One key direction is the exploration of novel preservation techniques that can extend the shelf life and enhance the quality of harvested produce. Nonthermal technologies, such as high-pressure processing, pulsed electric fields, and ultrasound treatment, have shown promise in maintaining the freshness and nutritional attributes of fruits and vegetables (Sun et al., 2011; Pan et al., 2020). These technologies can potentially replace traditional thermal treatments, offering more energy-efficient and environmentally friendly options. Another area of focus is the development of intelligent packaging systems that incorporate sensors, indicators, and active materials to monitor and regulate the post-harvest environment. These advanced packaging solutions can provide real-time information on the quality and freshness of the produce, detect spoilage factors, and release bioactive compounds to extend shelf life (López-Rubio et al., 2020; Singh et al., 2021). Additionally, integrating nanotechnology in packaging materials promises enhanced barrier properties and controlled release of antimicrobial agents, further contributing to post-harvest preservation (Chaudhry et al., 2018).

There is growing interest in understanding the molecular and genetic mechanisms underlying post-harvest processes. Advances in genomics, transcriptomics, proteomics, and metabolomics have enabled researchers to unravel the complex networks regulating fruit ripening, senescence, and post-harvest responses (Ding et al., 2019; Huang et al., 2021). This knowledge can be leveraged to develop targeted interventions, such as genetic modification or gene editing approaches, to improve post-harvest traits and reduce losses. However, along with these promising avenues, several challenges need to be addressed. Sustainable post-harvest practices that minimize waste, reduce energy consumption, and mitigate environmental impact are of utmost importance. Finding alternative solutions to synthetic chemicals for pest and disease management, such as biocontrol agents and natural compounds, is a critical area for further research (Adu-Gyamfi et al., 2020; Fuentes et al., 2021). Additionally, post-harvest research should consider the specific requirements and constraints of different crop types and geographical regions, ensuring the practical applicability and relevance of the developed technologies.

Challenges and opportunities and potential areas for enhancing tuber crop quality

Tuber crops, such as potatoes, sweet potatoes, yams and taro play a vital role in global food security. Enhancing tuber crop quality and extending their post-harvest shelf life are important challenges in ensuring food availability and reducing post-harvest losses. Here is some information on the challenges, opportunities, and potential areas for future investigation in these areas:

Challenges for enhancing tuber crop quality:

1. **Physiological Changes:** Tuber crops undergo various physiological changes during storage, including sprouting, weight loss, starch degradation, and accumulation of reducing sugars. These changes negatively impact quality and shelf life.
2. **Post-Harvest Losses:** Tuber crops are susceptible to damage during harvesting, handling, transportation, and storage. Mechanical injuries, diseases, and pests can lead to significant losses, reducing their market value.
3. **Sprouting and Dormancy:** Sprouting is a significant issue during storage, as it affects tuber crops' quality and nutritional value. Managing dormancy and preventing sprouting is crucial for maintaining their quality.
4. **Pathogen and Disease Control:** Tuber crops are prone to various pathogens and diseases, such as late blight in potatoes. Controlling these diseases is essential for preserving tuber quality and preventing post-harvest losses.

Opportunities for enhancing tuber crop quality:

1. **Breeding and Genetic Improvement:** Developing improved varieties with enhanced resistance to diseases, pests, and physiological disorders can improve tuber crop quality. Breeding programs can focus on traits like extended shelf life, reduced sprouting, and better storage characteristics.
2. **Pre-Harvest Factors:** Implementing appropriate pre-harvest practices, such as optimizing irrigation, nutrient management, and crop protection strategies, can positively impact tuber quality and post-harvest performance.
3. **Post-Harvest Technologies:** Utilizing post-harvest technologies like modified atmosphere storage, controlled atmosphere storage, and low-temperature storage can help extend the shelf life of tuber crops. These technologies slow down physiological processes and inhibit microbial growth.
4. **Integrated Pest Management (IPM):** Implementing IPM practices can effectively control pests and diseases while minimizing the use of chemical pesticides. IPM strategies include cultural practices, biological control agents, and resistant varieties.

Potential areas for future investigation on strategies for enhancing post-harvest quality and shelf life of tuber crops:

1. **Physiological Mechanisms:** Understanding the underlying physiological mechanisms involved in tuber crop quality changes during storage can provide insights into developing targeted strategies for maintaining quality and extending shelf life.
2. **Post-Harvest Treatments:** Investigating the effects of various post-harvest treatments, such as applying antioxidants, ethylene inhibitors, and plant growth regulators, can help identify effective methods for reducing sprouting, weight loss, and decay.
3. **Molecular Approaches:** Exploring the molecular mechanisms underlying tuber development, dormancy, and sprouting can lead to identifying critical genes and regulatory pathways. This knowledge can be used to develop molecular tools for improving tuber quality and storage life.
4. **Sustainable Packaging:** Researching sustainable packaging materials and technologies that minimize moisture loss, control gas exchange, and prevent mechanical damage can contribute to maintaining tuber quality during storage and transportation.
5. **Consumer Preferences and Market Demand:** Studying consumer preferences for tuber crop quality attributes, such as taste, texture, and nutritional value, can help guide breeding programs and post-harvest interventions to meet market demands.

Continued research and innovation in these areas can contribute to enhancing the quality, shelf life, and market value of tuber crops, ensuring their availability and reducing post-harvest losses

Conclusion

In conclusion, insights from physiological perspectives highlight critical strategies for enhancing the post-harvest quality and shelf life of tuber crops. Understanding factors such as respiration rates, ethylene production, and water loss is crucial in improving post-harvest management (Atanda et al., 2011). Implementing modified atmospheric packaging, controlled temperature and humidity conditions, and natural compounds can effectively extend shelf life. Further research and development are needed to optimize these strategies for practical application, reducing losses and increasing the market value of tuber crops.

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Performance of improved varieties of cassava in two agroecological units of Kerala

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Abstract

Cassava is an important source of energy in the diet of tropical countries of 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 and 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 Mattathur gram panchayat of Thrissur district which falls under Northern laterites Agro Ecological Unit 11 (AEU11) and Vellamunda panchayat of Wayanad district under Wayanad Central Plateau Agro Ecological Unit 21 (AEU21) 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, Thiruvananthapuram, Kerala. Hundred farmers were selected from Mattathur Krishi Bhavan of Thrissur district and fifty farmers from Vellamunda Krishi Bhavan of Wayanad district. Quality planting materials of improved varieties of cassava from ICAR-CTCRI viz., Sree Jaya, Sree Vijaya, Sree Pavithra, CTM 820 and CTM 806 were distributed to the farmers for cultivation in area of 10 cents of each, with a total area of 6 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 5.40 kg and 3.70 kg per plant with an average number of tubers 6.30 and 4.68 per plant in Mattathur and Vellamunda, respectively. Improved varieties of cassava produced significantly higher average tuber yield of 66.67 t ha⁻¹ and 45.68 t ha⁻¹ at Mattathur and Vellamunda, respectively. In both the locations, farmers could also produce 1.50 lakhs stems of improved varieties of cassava from 6 ha area within one season which were distributed to neighboring farmers and nearby districts for cultivation in an area of 60 ha.

Keywords: Cassava, quality planting material, multiplication, farmers

Introduction

Cassava is the fifth most important food crop in the world and was initially adopted as a popular famine reserve crop as it provided a more reliable source of food during

drought and hunger. It has emerged as both staple food and profitable cash crop of industrial significance in the world economy (Aerni, 2006). Cassava has enormous potential in India for poverty alleviation, food security

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and industrial use due to its ability to grow and yield well in marginal and wastelands. The crop is propagated vegetatively resulting in a low multiplication rate (1:10). Difficulty in transporting the planting materials to distant places due to bulkiness of planting material and the cassava mosaic disease infection are the major hindrance which prevent the spread of the 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 them the future crops. Inadequate availability of quality planting material of tuber crops continues to remain as a major stumbling block 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. 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). 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). Moreover, it is also used in various industries including starch and starch-derived products such as sago, textile, alcohol and high fructose-glucose syrups (Joseph et al., 2004; Yan et al., 2013). The present study was carried out with the objective of assessing the performance of improved varieties of cassava in two districts of Kerala along with multiplication of planting materials.

Material and Methods

Performance of improved varieties of cassava was studied at two distinct panchayats of Kerala. Mattathur gram panchayat of Thrissur district lies under Northern laterites Agro Ecological Unit 11 (AEU 11). This AEU area the climate is tropical humid monsoon type with mean annual temperature 27.5°C and rainfall ranging from 2795 to 3217 mm, laterite soils are the most extensive in the unit covering almost entire midlands. Vellamunda panchayat of Wayanad district of Kerala comes under Wayanad Central Plateau Agro Ecological Unit 21 (AEU 21). The climate is tropical humid monsoon type with mean annual temperature of 22.6°C and rainfall 2659 mm, upland soils are deep, acid clays and rich in organic matter and are suitable for cassava cultivation. The programme was implemented through Mattathur Krishi Bhavan of Thrissur district and Vellamunda Krishi Bhavan of Wayanad 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, Thiruvananthapuram, Kerala funded by the Department of Agriculture, Government of Kerala. The quality planting materials of improved varieties of cassava viz., Sree Jaya, Sree Vijaya, Sree Pavithra, CTM 820 and CTM 806 were distributed to farmers for planting in an area of 10 cents per each beneficiary, and thus covering a total area of 6 ha. A total of 100 farmers from Mattathur Krishi Bhavan area and 50 farmers from Vellamunda, Krishi Bhavan area were selected. Four skill-based training programme on scientific cultivation practices including large scale production of planting materials of cassava, agro techniques, organic farming, miniset quality planting material production (James George et al., 2004), seed treatment, value addition, plant protection measures, harvesting and seed certification standards were organized. Scientists and experts of ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram visited the farmers' fields periodically and given technical advice on intercultural operations, remedial measures for incidence of pest and disease, rouging of off types and disease infected plants etc. The yield data were recorded at the time of harvest.

Result and Discussion

Based on random sampling, an average cassava tuber yield of 66.67 t ha⁻¹ was recorded at Mattathur panchayat area. Cassava tuber yield varied from 2.50 to 12.0 kg per plant at Mattathur with an average tuber yield of 5.40 kg per plant. Number of tubers ranged from 3 to 12 per plant with an average of 6.30 per plant at Mattathur panchayat area (Table 1 and Fig. 1).

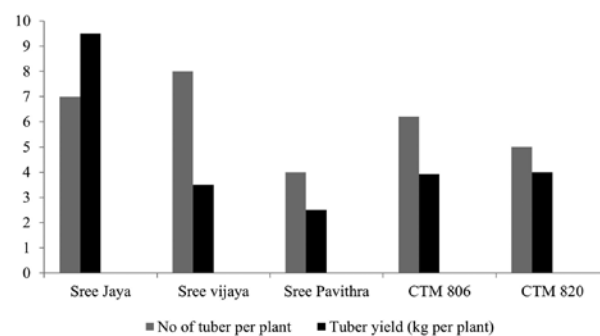


Fig. 1. Tuber yield and number of tubers per plant for different cassava varieties at Mattathur

Based on random sampling, improved varieties produced higher average tuber yield of 66.67 t ha⁻¹ by improved varieties along with better intercultural management conditions in Mattathur panchayat. The farmers got an average tuber yield ranging between 2.0 to 5.7 kg per plant and number of tuber 2-8 per plant with an average yield of 3.70 kg per plant and average tuber number of 4.67 per plant and total tuber yield 45.68 t ha⁻¹ at Vellamunda, Krishi Bhavan area of Wayanad district (Table 2 & Fig. 2)

Table 1. Yield Performance of cassava varieties in the farmers' field at Mattathur panchayat, Thrissur

Sl. No.	Name of the farmer	Name of the village	No. of tubers plant ⁻¹	Tuber yield (kg plant ⁻¹)	Variety
1.	Ms. Sarada	Vellikulangara	7	12.0	Sree Jaya
2.	Mr. Asokan	Kodali	7	7.0	Sree Jaya
3.	Mr. Satheesan	Mattathurkunnu	6	4.2	CTM 806
4.	Mr. Sukumaran	Mattathurkunnu	5	4.0	CTM 820
5.	M. Sukumaran	Mattathurkunnu	12	4.5	Sree Vijaya
6.	Ms. Ajithakumari	Chettichal	4	2.5	Sree Vijaya
7.	Ms. Jayalekshmi	Chettichal	6	3.5	CTM 806
8.	Ms. Mary	Padi	3	2.5	Sree Pavithra
9.	Mr. Sooraj	East Kodali	7	4.0	CTM 806
10.	Mr. Shajahan	Padi	6	4.0	CTM 806
Mean			6.3	5.4	

*Mean number of tuber per plant - 6.3, Mean tuber yield per plant - 5.4 kg
Average tuber yield per ha - 66.67 t ha⁻¹, Average tuber yield per unit area - 2,666 kg (0.10 acre)

Table 2. Yield Performance of cassava varieties in the farmers' field at Vellamunda Panchayat, Wayanad district

Sl.No	Name of the farmers	Name of the village	No. of tubers plant ⁻¹	Tuber yield (kg plant ⁻¹)	Variety
1.	Mr. Santha Ajeesh	Nellicachal	6	4.500	Sree Jaya
2.	Mr. Ravi	Nellicachal	4	3.000	Sree Jaya
3.	Mr. Lekshmi	Nellicachal	4	2.500	Sree Jaya
4.	Mr. Kurumbi	Nellicachal	5	3.500	Sree Vijaya
5.	Mr. Chandran	Karakkamala	3	2.000	Sree Vijaya
6.	Mr. Ratheesh	Nellicahal	6	3.000	Sree Jaya
7.	Mr. Mallan	Karakkamala	3	2.000	Sree Jaya
8.	Mr. Balakrishnan	Karakkamala	2	1.600	Sree Jaya
9.	Mr. Santha	Karakkamala	6	5.700	Sree Jaya
10.	Mr. Korumbi	Karakkamala	5	4.800	Sree Pavithra
11.	Mr. Balan	Karakkamala	8	4.000	Sree Pavithra
12.	Ms. Leela	Karakkamala	4	3.000	Sree Jaya
Mean			4.67	3.70	

*Mean number of tubers per plant - 4.67, Mean tuber yield per plant - 3.70 kg
Average tuber yield per ha - 45.68 t ha⁻¹, Average tuber yield per unit area - 1,850 kg (0.10 acre)

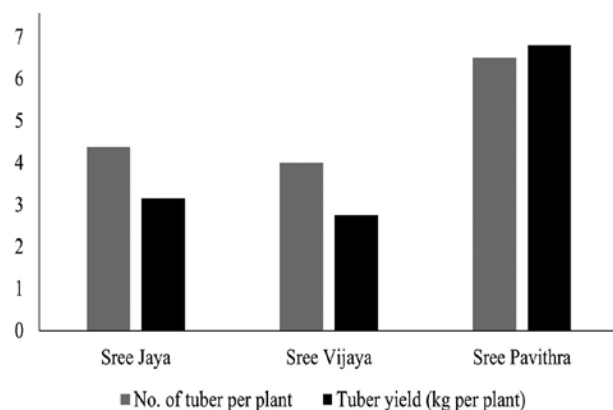


Fig.2. Tuber yield and number of tubers per plant for different cassava varieties at Vellamunda

In general, performance of improved varieties of cassava was better in AEU 11 and there was a decline in tuber yield by 31% in AEU 21. Sree Jaya performed better in location (12 kg plant⁻¹) followed by Sree Vijaya (4.5 kg plant⁻¹). The low temperature prevailed during the cultivation at Wayanad might be the major reason for the lesser yield. Taking into consideration the cost of cassava stems, transportation, field preparation, planting and other related expenses, the total cost of cultivation was estimated as ₹8000 for an area of 10 cents. However, planting materials and cultivation cost expenses were distributed to beneficiary farmers from ICAR-CTCRI, Thiruvananthapuram under the 'Development of tuber crops' scheme. On average, farmers got tuber yield of 5.40 kg per plant at Mattathur panchayat in

Thrissur district. In the case of Vellamunda panchayat of Wayanad district, farmers got an average yield of 3.70 kg per plant. At the time of harvesting, tuber price in the local markets was 15-20 per kg. Average B:C ratio was worked out as 4.64:1 at Mattathur and 3.63:1 at Vellamunda (Table 3).

Table 3. Economics of cassava cultivation in farmers' field (0.10 acre)

Item	Mattathur	Vellamunda
Cultivation expenses including cost of planting materials (₹)	8,000	8,000
Tuber yield, kg (0.10 acre)	2670	1850
Gross returns 20 per kg for tuber	53400	37,000
Net return@ ₹	45400	29,000
B:C Ratio	6.67:1	4.63:1

Conclusion

The improved varieties of cassava were raised based on the ICAR-CTCRI package of practices given to farmers in the training programme. The results revealed that the average yield was higher in Mattathur (66.67 t ha⁻¹) as compared to Wayanad (45.68 t ha⁻¹). The average tuber yield was 66.67 t ha⁻¹ and 45.68 t ha⁻¹ in Mattathur and Vellamunda panchayat, respectively. Accordingly, the net income benefit-cost ratio from cassava crop was 6.67:1 in the Mattathur panchayat of Thrissur district and 4.62:1 in the Vellamunda panchayat of Wayanad district of Kerala. Due to implementation of this programme, farmers got sufficient good quality planting material of cassava which is fast spreading in the neighborhood area and increased the income generation from cassava.

Farmers could produce 1.50 lakhs cassava stems of improved varieties of cassava from 6 ha area within one season which were distributed to neighbourhood farmers for cultivating in an area of 60 ha.

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Genetic variability for different quantitative characters in colocasia (*Colocasia esculenta* var. *antiquorum*.)

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Abstract

An experiment was conducted with nine *Colocasia* (*Colocasia esculenta* var. *antiquorum* (L.) Schott.) genotypes to evaluate the genetic variability for different quantitative characters. The experiment was conducted using a Randomized Complete Block Design with three replications. The genetic parameters between yield and yield contributing characters of different *Colocasia* genotypes were studied. Analysis of variance showed significant variation among the genotypes for all tested characters. The highest total yield was recorded in Indira Arvi-1 (29.54 t ha⁻¹), which was followed by TTr 17-1 and TTr 17-12 (25.31 t ha⁻¹ and 18.27 t ha⁻¹, respectively). Corm weight showed the highest genotypic and phenotypic variance (71.52 and 72.41), whereas the number of leaves showed the lowest one (14.95 and 18.72). High value of heritability was observed for all the characters except number of leaves per plant. Genetic advance as percent of mean was reported highest for total yield, yield per plant, weight of corm and plant height. The genotypes exhibited a wide range of variability for all the traits studied.

Keywords: *Colocasia*, Genetic variability, Heritability, Genetic advance, Corm yield

Introduction

Colocasia (*Colocasia esculenta* var. *antiquorum* (L.) Schott) is one of the most popular and extensively consumed tubercrops grown world wide due to its acclimatization to a wide variety of environments. It is also known as taro and arvi. It belongs to the family Araceae and is a native of South-east Asia. It is grown throughout the tropics for its edible corms and leaves and is believed to be one of the earliest cultivated tuber crops in the world (Kuruvilla and Singh, 1981). Food and Agriculture Organization (FAO) reported that taro production has doubled over the past decade (FAOSTAT, 2000) and is now the fifth most-consumed root vegetable world wide.

Success of plant breeding depends upon the nature and magnitude of variability present in the different genotype. Moreover, evaluating the heritable and non-heritable aspects of overall unviability will hold significant importance in selecting appropriate breeding methodologies. Corm yield is a quantitative character, which is influenced by a number of yield contributing characters such as plant density, soil quality, cultural and farming practices, climatic conditions and so on. Selection for corm and cormel yield, the complex interrelationship between the yield contributing characters usually shows a complex chain of interrelating relationship. In Chhattisgarh, colocasia is grown during the rainy and summer seasons. It is one of the most

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important tuber crops of Chhattisgarh. However, the yield of Colocasia in Chhattisgarh is not satisfactory enough in comparison with other Colocasia growing states. Despite tremendous potentialities, aroids are running in vulnerable condition without being properly and scientifically evaluated. Hence, the present study was planned to evaluate genetic parameters for corm yield and yield contributing characters to find out and establish suitable selection criteria for higher corm yield through the study of variability. The main objectives were to estimate the variation through in-depth study on gross morphological characters, the phenotypic and genotypic variability present in different characters, contributing to yield per plant and to estimate the heritability and genetic advance for yield per plant and its components.

Materials and Methods

The study was carried out during the *Kharif* season of 2020-21 under the All India Coordinated Research Project on Tuber Crops at S. G. College of Agriculture and Research Station, IGKV, Jagdalpur, Chhattisgarh. The experimental material comprised of nine genotypes of Colocasia (TTr 17-1, TTr 17-2, TTr 17-3, TTr 17-5, TTr 17-8, TTr 17-12, TTr 17-13, and the two check varieties, viz., Sree Rashmi and Indira Arvi-1). The experiment was laid out in a randomized block design with three replications at the spacing of 60 cm between rows and 45 cm between plants. A plot size of 3m × 3m was kept for each genotype. All the recommended cultural practices were followed to grow a healthy crop. Data was recorded on five randomly selected plants for seven characters viz., plant height (cm), number of leaves per plant, number of cormel, weight of corm (g), weight of cormels (g), yield per plant (g) and total yield (corm + cormel) (t ha⁻¹). The data were subjected to statistical and biometrical analysis (Singh and Chaudhary, 1985).

The coefficient of variation for different characters was estimated using the formula suggested by Burton (1952). The estimates of genotypic and phenotypic coefficient of variance were classified as low (less than 10%), moderate (10 to 20%) and high (more than 20%) as suggested by Sivasubramanian and Madhavamenon (1973). The expected genetic advance was calculated using the formula given by Johanson et al., (1955). Heritability in broad sense (h^2_{bs}) was calculated as per the formula suggested by Burton and De Vane (1953).

Results and Discussion

The analysis of variance of all the characters under study is presented in Table 1. This revealed that the mean sum of squares due to genotypes was highly significant for all characters. This is an indication of the existence of sufficient variability among the genotypes for total yield and its components traits. Significant mean sum of squares due to total yield (corm + cormel) and attributing characters revealed existence of considerable variability in material studied for improvement for various traits. These findings are in general agreement with the findings of Paul et al., (2011) and Cheema et al., (2007).

The mean performance and genetic variability were estimated and presented in Tables 2 and 3. The significantly higher total yield (corm + cormel) was recorded in the genotype Indira Arvi-1 (29.54 t ha⁻¹), followed by TTr 17-1 (25.31 t ha⁻¹) and TTr 17-12 (18.27 t ha⁻¹). Maximum plant height was recorded in Indira Arvi -1 (105.66 cm) followed TTr 17-1 (52.10 cm) and TTr 17-12 (48.30 cm). Maximum number of leaves per plant was recorded in Indira Arvi-1 (9.84) followed by TTr 17-5 (9.12). Maximum number of cormels per plant, weight of cormels and weight of corms recorded in Indira Arvi-1 was 14.73, 539.96 g

Table 1. Analysis of variance for corm yield and its component characters in Colocasia

Sl. No.	Character	Mean sums of square		
		Replication	Treatment	Error
1	Plant height (cm)	6.93	1274.99**	215.42
2	Number of leaves per plant	0.16	4.86**	0.77
3	Number of cormels	0.05	41.50**	1.26
4	Weight of corm (g)	47.53	21936.62**	181.25
5	Weight of cormels (g)	374.84	56780.70**	1419.07
6	Yield per plant (g)	165.22	123579.05**	2045.02
7	Total yield (t ha ⁻¹)	0.225	169.48**	2.8

** : Significant at 1%

and 257.7 g, respectively followed by TTr 17-1 (14.33, 486.42 g and 196.89 g, respectively). A wide range of variation was recorded for plant height, corm weight, cormel weight and total yield, which indicated that there is better scope for selection for the improvement of these characters. Pandey et al., (1996) observed wide range of variability among 31 genotypes for yield plant⁻¹, weight of mother cormels and weight of cormels. These findings are in close proximity with the results of Cheema et al., (2007) who reported variability for number of leaves per plant, number of cormels per plant, corm weight and yield per plant. Similar findings were reported by Solanki et al., (2001), Mukherjee et al., (2003) and Singh et al., (2003).

Maximum values for genotypic (71.52%) and phenotypic (72.41%) coefficient of variation were observed for

weight of corms followed by total yield (47.12 and 48.02%, respectively) and yield per plant (46.72 and 47.88%, respectively). The least genotypic and phenotypic coefficient of variation was observed for number of leaves per plant (14.95 and 18.72%). Phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all the traits indicating that environmental factors were influencing their expression.

Wide difference between phenotypic and genotypic coefficient of variations indicated their sensitivity to environmental fluctuations whereas, narrow difference showed less environmental interference on the expression of these traits. The traits which showed high phenotypic and genotypic coefficient of variations are of economic importance and there is scope for improvement of these traits through selection. These characters implied their

Table 2. Mean performance for corm yield and its components in Colocasia

Genotype	Character						
	Plant height (cm)	No. of leaves per plant	No. of cormels	Weight of corm (g)	Weight of cormels (g)	Yield per plant (g)	Total yield (t ha ⁻¹)
TTr 17-1	52.10	6.50	14.33	486.42	196.89	683.31	25.31
TTr 17-2	41.11	6.61	11.89	363.55	45.77	409.33	15.16
TTr 17-3	43.87	5.78	13.78	279.77	87.11	366.88	13.59
TTr 17-5	45.37	9.12	12.89	321.33	85.55	406.88	15.07
TTr 17-8	38.67	8.17	5.44	109.34	52.97	162.31	6.01
TTr 17-12	48.30	8.55	5.33	259.11	234.2	493.30	18.27
TTr 17-13	43.00	7.66	9.33	271.55	50.66	322.21	11.93
Sree Rashmi	45.67	8.05	8.01	174.38	60.74	235.12	8.71
Indira Arvi-1	105.66	9.84	14.73	539.96	257.7	797.66	29.54
Mean (x)	51.53	7.81	10.64	311.71	119.07	430.78	15.95
SEm±	2.12	0.48	0.65	21.75	7.77	26.11	0.97
CD (p=0.05)	6.35	1.42	1.94	65.01	23.23	78.05	2.89

Table 3. Genetic parameters for yield and its attributing characters in Colocasia

Sl.No.	Character	Mean	Range		Coefficient of variation (%)		Heritability (h ² , %)	GA as percent of mean
			Min	Max	GCV	PCV		
1	Plant height (cm)	51.53	38.67	105.66	39.83	40.46	96.89	80.77
2	No. of leaves per plant	7.81	9.84	6.50	14.95	18.72	63.75	24.59
3	No. of cormels	10.64	5.33	14.33	34.43	36.01	91.39	67.8
4	Weight of corm	119.07	45.77	257.70	71.52	72.41	97.56	45.52
5	Weight of cormels	311.71	109.34	539.96	43.46	46.11	92.82	86.25
6	Yield per plant (g)	430.78	162.31	797.78	46.72	47.88	95.09	93.90
7	Total yield (t ha ⁻¹)	15.95	6.01	29.54	47.12	48.02	96.19	94.64

relative resistance to environmental variation. These findings are in consonance with Mukherjee et al., (2003), Cheema et al., (2007), Devi and Singh (2019).

Heritability and Genetic Advance

In the present investigation, heritability estimates in broad sense are depicted in Table 3. High estimates for heritability were exhibited by all the characters except number of leaves per plant (63.75%), which showed moderate estimates of heritability. Weight of corm (97.56%), plant height (96.89%), total yield (96.19%), yield per plant (95.09%), weight of cormels (92.82%) and number of cormels (91.39%) showed high heritability. The characters which showed high value of heritability demonstrated that they were least influenced by environmental changes and selection based on phenotypic performance would be reliable. Similar results were also reported by Pandey et al., (1996), Singh et al., (2003) and Paul et al., (2011). On the other hand, high heritability coupled with high genetic advance was observed for all the characters except number of leaves per plant. Weight of corm (97.56% and 45.52%), plant height (96.89% and 80.77%), total yield (96.19% and 94.64%), yield per plant (95.09% and 93.9%), weight of cormels (92.82% and 86.25), number of cormels (91.39% and 67.8%) exhibited high heritability with high genetic advance indicating that most likely the heritability is due to additive gene effects and selection may be effective. Therefore, selection based on phenotypic performance of these traits would be effective to select desirable plant types. Pandey et al., (1996) observed high heritability coupled with high genetic advance for weight of mother cormels, weight of cormels and yield per plant. Similar results were also reported by Paul et al., (2011) who observed high heritability with moderate to high genetic advance for plant height, petiole length, leaf length, stolon weight, total stolon weight, stolon length and corm length. Number of leaves per plant showed moderate heritability (63.75%) with low value of genetic advance (24.59%). Characters which showed moderate to low heritability coupled with moderate to low genetic advance as percentage mean indicated the role of non-additive genetic variance in their expression.

Conclusion

Nine *Colocasia* (*Colocasia esculenta* var. *antiquorum* (L.) Schott.) genotypes were evaluated for the genetic variability for different quantitative characters. The study revealed sufficient genetic variability for quantitative

traits among the genotypes, which can be exploited for varietal improvement and can be further used as a source material to develop promising varieties in colocasia.

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Harnessing the diversity of bacterial endophytes isolated from wild and cultivated taro plants against *Phytophthora colocasiae*

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Abstract

Taro (*Colocasia esculenta* (L.) Schott), a tuber crop which belongs to Araceae family is an important staple or subsistence crop for millions of people in developing countries. The crop capitulates to several fungal, bacterial, and viral diseases as well as some diseases of uncertain etiology. Taro leaf blight, a threatening disease of taro caused by *Phytophthora colocasiae* is associated with 90% and 50% loss in leaf and corm yield of taro, respectively. The preventive measures used by crop rotations and the use of improved disease resistant varieties have failed to completely eradicate the disease. Chemical fungicides are not only costly but have harmful effects on humans and the environment. Endophytes with antifungal activity can be exploited as excellent biocontrol agents against phytopathogenic fungi. Hence, this study was centred on evaluating the antagonistic activity of endophytic bacteria and fungi associated with wild and cultivar taro plants itself against *Phytophthora colocasiae*. The study involves isolation of endophytic bacteria and evaluation of antagonism against the pathogen using *in vitro* dual culture method. A total of 97 bacterial endophytes were isolated from taro plants and they were evaluated for their antagonistic activities against *Phytophthora colocasiae*. The *in vitro* study indicated that among the bacterial isolates, KV9 showed the highest antagonistic activity of $84.07 \pm 1.04\%$. This research study demonstrates that these endophytes can be exploited to create a promising biocontrol agent against *P. colocasiae* in the taro field.

Keywords: Taro, *Phytophthora colocasiae*, endophytes, biocontrol, antagonism, dual culture method

Introduction

Taro (*Colocasia esculenta* (L.) Schott), is one of the important tropical tuber crops which belongs to the family Araceae. It is widely cultivated for its edible underground corms, which is the main source of carbohydrates, starch, ash, phytochemicals, vitamins etc and leaves as a staple food for millions of people in developing countries like Asia, Africa, and Central America (Nath et al., 2012, Rashmi et al., 2018). Two commonly cultivated varieties of taro are *Colocasia esculenta* var. *esculenta* and *Colocasia esculenta*

var. *antiquorum* (Ahmed et al., 2020). Taro ranks second among the staple root crops in terms of consumption after sweet potato with about 12 million tonnes produced globally from about 2 million hectares with an average yield of 7 tonnes per hectare (FAOSTAT 2021; <http://faostat.fao.org>). Although taro is planted for the corm, the leaves, stem, and flowers are all edible and have an exceptional nutritional value.

The crop capitulates to several fungal, bacterial, and viral diseases as well as some diseases of uncertain etiology.

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Major among them, *Phytophthora colocasiae* Rac., an oomycete, is primarily a foliar pathogen which affects taro and causes one of the most ruinous diseases of taro called taro leaf blight (TLB). The initial symptoms of TLB are shown on leaves as small purple or brown water-soaked speckles which enlarge to form yellow marginated dark brown lesions which cause defoliation and ultimately lead to death of crop. The disease is associated with 95% and 50% loss in leaf and corm yield of taro, respectively (Singh et al., 2012). Despite the normal 40 days life span of healthy taro leaves, infected leaves devastate within 20 days. The severity of leaf blight is shown maximum at areas having high relative humidity and frequent rainfall compared to warmer areas. Under cloudy weather conditions with patchy rains and temperature around 28°C, disease spreads at tremendous speed and the entire field gives a blighted appearance (Jackson et al., 1980; Misra et al., 2008; Singh et al., 2012). As taro is a vegetatively propagated crop, TLB is frequently spread through the usage of planting materials infested with *P. colocasiae* sporangia and zoospores. The pathogen could carry over to the next season through mycelium in dead and dying plant tissues and infected corms, and through encysted zoospores or as chlamydospores in soil (Okereke, 2020; Nelson et al., 2011).

Diverse approaches have been employed for the sustainable management of TLB, using resistant cultivars developed in India, viz., Muktakeshi, Bhu Kripa, Thamarakannan (ICAR-CTCRI, 2020), Poonam pat, Sakina V and by using chemical fungicides etc. (Misra et al., 2008). Metalaxyl and Mancozeb based phenylamide fungicides are the most effective and commonly used ones by the farmers against TLB in India (Nath et al., 2013). Although this strategy has produced encouraging results, phytotoxicity and chemical residues are significant issues that put the environment and human health at risk. Also, fungicides are too costly to be affordable by the marginal farmers and the development of resistant strains against the fungicide is another threat. Thus, the need for an alternative for managing taro leaf blight becomes inevitable. Recently the trend of using chemical free products has been increased. Among those, bio-control agents are getting special attention by scientists as they are eco-friendly, economical, sustainable, and potent alternative to control many virulent plant pathogens (Hong et al., 2021). One of the most successful biological control methods in agriculture is the use of fungal and bacterial endophytes (Moise et al., 2018).

Endophytes are microorganisms, most commonly bacteria and fungi, which colonize the internal tissue of living plants without causing any harm, at least while they are in the endophytic stage of their life cycle (Kushwaha et al., 2020). Numerous studies have revealed an enormous diversity of endophytic bacteria in plant systems and

have pinpointed their potential contribution to disease resistance and plant growth promotion (Martinez et al., 2017). Bacterial endophytes have been noticed to impede the onset of disease by enabling the de novo synthesis for novel phytochemicals and secondary metabolites which may have antimicrobial, antifungal, anticarcinogenic, immune-suppressant or antioxidant activity. Harnessing endophyte-plant interactions could enhance plant health and be an essential aspect of low-input sustainable agriculture applications (Ryan et al., 2008).

The goal of this study is to identify and describe bacterial endophytes that were isolated from taro plants and to investigate the potential of these endophytes to act as a biocontrol agent against *Phytophthora colocasiae*, which is responsible for causing taro leaf blight disease.

Materials and Methods

Isolation of fungal pathogen, *Phytophthora colocasiae*

Leaf blight infected leaf samples of different taro plants were collected from various regions of Kerala, India for the isolation of fungal pathogen *Phytophthora colocasiae*. Infected leaf samples were cut into small bits such that the bits contain both infected region and healthy region and washed with sterile distilled water. Surface sterilization was carried out with back-to-back washing using 3% sodium hypochlorite solution for 2 min, 70% ethanol for 1 min., followed by three consecutive washes using sterile distilled water (Anjum and Chandra, 2015). Sample bits were allowed to dry using Whatman filter paper and transferred aseptically to potato dextrose agar medium with 100 µl ampicillin. The Petri plates were incubated at 28±2°C in the BOD for 4-6 days. Mycelia from the growing verge were subcultured on carrot agar medium plates. The pure cultures were primarily confirmed by microscopically examining the mycelium and sporangia. The cultures were maintained on carrot agar slants at 4°C for further study. Pathogenicity assay was done by detached leaf assay to choose the most virulent pathogen strains from the isolated ones as per Nath et al., (2016).

Confirmation of pathogen using species specific primers

The total genomic DNA was extracted from the fungal pathogen by Cetyl Trimethyl Ammonium Bromide (CTAB) method (Karthikeyan et al., 2010). The genomic DNA was amplified using universal fungal specific ITS1 and ITS4 primer pairs and *Phytophthora colocasiae* specific primer pairs such as PCSP-RF and PCSP-RR. PCR assays were carried out in an automated temperature cycling device (Agilent Tech). The amplified PCR products were size fractionated on a 1.5% agarose gel stained with ethidium bromide and the image was analysed by Gel Doc System (Alpha Innotech Corporation, San Leandro, CA, USA).

Collection of taro plant samples for bacterial endophyte isolation

For the isolation of bacterial endophytes, healthy wild and cultivable taro plant samples were collected from fourteen different regions of Kerala. The uprooted plant samples were carried over to laboratory in sterile polythene sampling bags and used for further experiments.

Isolation of bacterial endophytes

Healthy tissues of leaves, petiole, corm, and roots of taro samples were used for isolation of bacterial endophytes. They were washed separately under running tap water to remove adhering soil particles and dirt. Each of the plant parts were then excised inside the laminar flow, into 1-2 cm bits using a sterile scalpel. These samples were surface sterilized using 3% sodium hypochlorite solution for 2 min, 70% ethanol for 1 min, followed by washing three times with sterile distilled water and the water in surface sterilized samples was removed with filter paper (Araujo et al., 2002, Anjum and Chandra, 2015). Each bit was placed on nutrient agar medium plates with three replications. By imprinting cultured aliquots of water from the most recent rinse onto nutrient media, the surface sterilisation technique was validated. They served as the control plates. The inoculated plates were incubated at $28 \pm 2^\circ\text{C}$ in a growth incubator for 24 to 72 h and observed. The individual bacterial colonies with visible differences in their morphology were sub-cultured in nutrient agar medium to maintain the pure cultures. Finally, all the purified endophytes were stored at 4°C until they were employed. All chosen isolates were subcultured in nutrient agar slants.

In vitro screening of bacterial endophytes for antagonistic activity against *P. colocasiae*

Isolated bacterial endophytes were assessed for their antifungal activity against *P. colocasiae* by modified dual culture technique on potato carrot agar plates (Shastri et al., 2020). After standardisation, potato carrot agar plates were made by potato dextrose agar and carrot agar in the ratio 1:3. A small circular mycelial plug of 4mm of *P. colocasiae* taken from an actively growing 7-day old culture on carrot agar plate was placed at the centre of the sterile 90 mm plate containing potato carrot agar. Simultaneously, the endophytic bacterial strains were rectilinearly streaked 30 mm away from fungal plug on opposite sides. The fungal culture grown on the potato carrot agar plate without any bacterial isolate served as control. The above setup was done in three replications. The plates were incubated for 6-8 days at $28 \pm 2^\circ\text{C}$ in BOD incubator. The radial growth of *P. colocasiae* mycelium was measured, and the percentage inhibition of mycelium radial growth of *P. colocasiae* over control was calculated with the following formula.

$$\text{Percentage of mycelial growth inhibition} = \frac{(C-T)}{C} \times 100$$

Where C was the radial growth (mm) of the control mycelium colony and T was the radial growth (mm) of the mycelium growing in presence of antagonist endophytic bacterial isolate.

Statistical analysis

The data were analysed wherever necessary using the free online software, (<https://sreejyothi.shinyapps.io/agrianalyticsr>) developed by ICAR-CTCRI, Thiruvananthapuram, Kerala, India and the treatments were compared.

Results and Discussion

A total of fifteen isolates of *P. colocasiae* were obtained from diseased taro leaf samples collected from different regions of Kerala, ICAR-CTCRI (Table 1). The isolates were tentatively recognised as *P. colocasiae* based on morphological traits such as colony morphology and sporangial characteristics (Fig. 1). According to Misra et al., (2008), the mycelium of *P. colocasiae* are hyaline, coenocytic, aseptate and the sporangia are elongated, slender and narrow ended.

Table 1. Different isolates of *Phytophthora colocasiae*

Sl. No.	Isolate code	Location	District/Sampling site
1	PCKA	Kerala	Thiruvananthapuram/ Vamanapuram
2	PCTH	Kerala	Kollam/Thalavur
3	PCSA	Kerala	Kollam/Sadanandapuram
4	PCCH	Kerala	Thiruvananthapuram/ Cheruvakkal
5	PCPA	Kerala	Thiruvananthapuram/Palode
6	PCCTA	Kerala	Block II/ICAR-CTCRI Field
7	PCCTB	Kerala	Block I/ICAR-CTCRI Field
8	PCHK	Kerala	Kollam/Kottarakkara
9	PCNA	Kerala	Malappuram/ Nilambur
10	PCPB	Kerala	Idukki/Kumily
11	PCKP	Kerala	Thrissur/Punna
12	PCSD	Kerala	Wayanad/Sultanbathery
13	PCNY	Kerala	Thiruvananthapuram/ Neyyatinkara
14	PCRM	Kerala	Kollam/Kulathupuzha
15	PCTL	Kerala	Kollam/Thenmala

The results of the pathogenicity assay are summarized in Table 2. All the isolates were virulent and reproduced serious infection on detached taro leaf discs except those isolated earlier. There were no lesions in the control leaf discs (Fig. 2). The isolates-initiated lesion development after 1-4 days of inoculation and the inoculated sites showed water-soaked lesions at the beginning which turned brown upon progression of the disease. There was a significant difference in the lesion diameter among the isolates (Fig. 3). The isolate PCHK which exhibited the highest virulence was used further for *in vitro* screening.

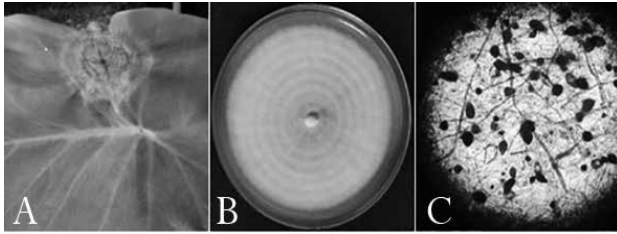


Fig.1. (a) Taro leaf blight symptom, (b) Eight days old culture of *P. colocasiae* on carrot agar medium, (c) Sporangia of *P. colocasiae* observed under 40×

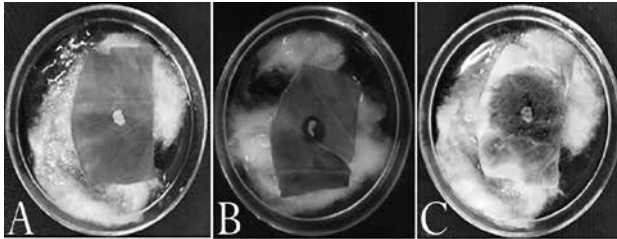


Fig. 2. Pathogenicity assay on detached taro leaves (a) 1st day of *P. colocasiae* mycelial plug inoculation. (b) Lesion appeared on 2 days after inoculation (c) Fully infected leaf on 5 days after inoculation

Table 2. Pathogenicity assay by different *P. colocasiae* isolates

Isolate code	Days taken for lesion developed	Lesion diameter (cm)
PCKA	1	4.53±0.06
PCTH	4	2.02±0.13
PCSA	2	4.00±0.20
PCCH	2	3.80±0.30
PCPA	3	3.33±0.29
PCCTA	1	4.82±0.08
PCCTB	4	2.47±0.06
PCHK	1	5.33±0.20
PCNA	2	3.63±0.15
PCPB	2	3.58±0.14
PCKP	3	3.33±0.14
PCSD	3	3.53±0.15
PCNY	2	3.72±0.20
PCRM	3	3.63±0.12
PCTL	4	3.13±0.23

CTAB method was used for the isolation of DNA from *P. colocasiae* isolates. The extracted genomic DNA was run on 1% agarose gel and visualized in Alpha Imager to observe the bands. The concentration of DNA obtained was 380 ng μL^{-1} and absorbance ratio were 1.6. The *P. colocasiae* specific primer pairs PCSP-RR and PCSP-RF successfully amplified all the *P. colocasiae* isolates and yielded an amplicon of size 206 bp approximately when resolved on 1.5% agarose gel.

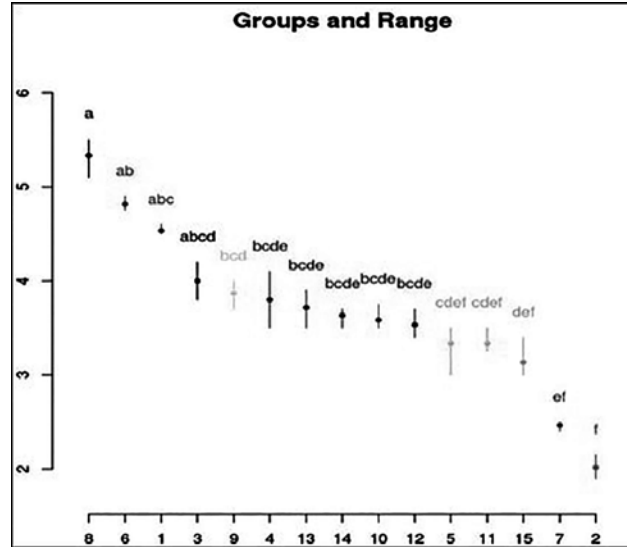


Fig. 3. Multiple comparison plot of virulence of *P. colocasiae* isolates

Endophytic microorganisms have attracted interest in agriculture in recent years due to their prospective impact on crop growth promotion, biocontrol, and disease resistance against diverse phytopathogens (Dwibedi et al., 2022). To obtain a broad diversity of endophytes, wild and cultivated kinds of taro plant samples have been analysed in this work to isolate diverse endophytes and investigate various functional features. Twelve taro plant samples were collected from different parts of Kerala, among them each seven were wild and cultivable taro plants. Root, corm, petiole, and leaves of the taro plants were used for the isolation of bacterial endophytes. The presence and diversity of bacterial endophytes identified from wild and cultivated taro types under natural circumstances are significant because these endophytes can benefit plant health. A total of 97 distinct bacterial endophytes were successfully isolated from roots, corms, petioles, and leaves from wild and cultivated varieties of taro plant samples, of which 33 from root, 27 from corm, 17 from petiole and 20 from leaves (Table 3).

One of the key and essential phases in the isolation approach is effective sterilisation and media selection (Shastri et al., 2020). As there was no growth on the control plates, the isolates can be confirmed as endophytic bacterium of taro.

Endophytic bacterial diversity varies significantly within different plant tissues such as root, corm, petiole, and leaf and between wild and cultivated varieties of plants. Endophytic diversity is greater in root tissues when compared to corm, petiole, and leaf tissue in both types (Fig. 4). The abundance of bacteria in root tissues may be owing to their ability to produce root exudates that promote the proliferation and colonization of bacteria (Karnwal and Dohroo, 2018).

Table 3. Distribution of bacterial endophytes in various plant tissues

Sl. No.	Place of sample collection	Wild/Cultivar	Number of bacterial endophytes			
			Root	Corm	Petiole	Leaf
1	Azhoor/ Pathanamthitta	Cultivated	2	1	2	0
2	Kariavattom/Thiruvananthapuram	Wild	4	2	5	2
3	Kumily/Idukki	Cultivated	2	1	1	2
4	Mavelikkara/Alappuzha	Wild	3	3	2	1
5	Ottapalam/Palakkad	Cultivated	1	2	1	0
6	Pala/Kottayam	Cultivated	2	2	0	0
7	Palode/Thiruvananthapuram	Cultivated	3	2	1	3
8	Periya/Kasaragod	Wild	3	2	1	3
9	Punna/Thrissur	Wild	4	3	0	2
10	Sultanbathery/Wayanad	Wild	3	4	1	3
11	Thenmala/Kollam	Wild	3	2	1	1
12	Ulloor/Thiruvananthapuram	Cultivated	3	3	2	3

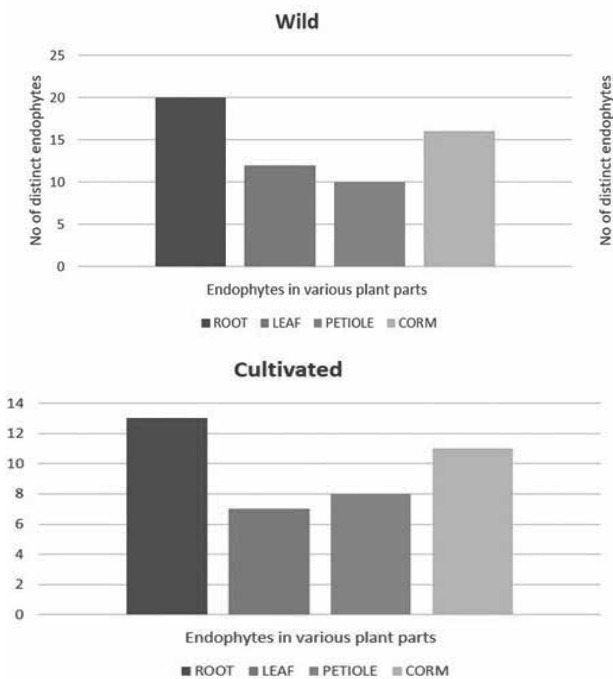


Fig. 4. Number of distinct endophytic bacteria isolated from various plant tissues of wild and cultivated taro samples

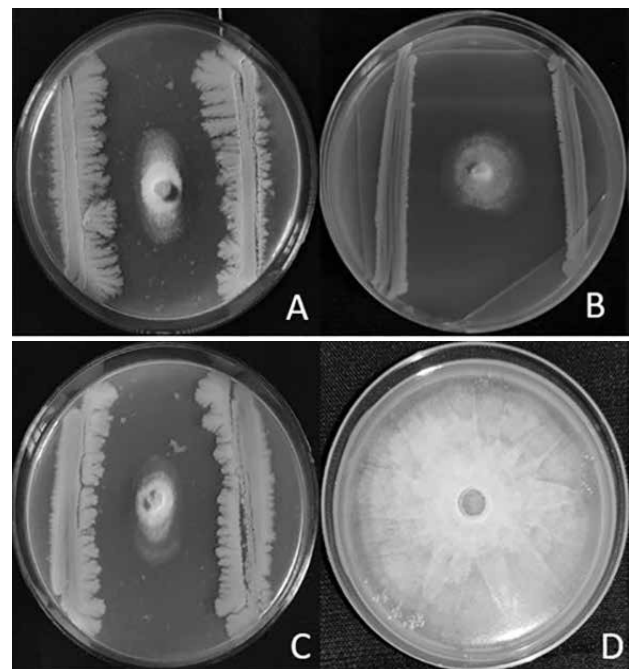


Fig. 5. Antagonistic activity of bacterial endophytes (A) PA3, (B) UL4, (C) KV9, (D) Control plate

In vitro screening of antagonistic activity of ninety-seven bacterial isolates by dual culture assay against *P. colocasiae* showed that seventy-three endophytic bacteria exhibited antagonistic activities against *P. colocasiae* in varying degrees (Table 4 and Fig. 5). Among those, 55% (40 bacterial endophytes) were from wild taro plant samples and rest (33 bacterial endophytes) from cultivated taro plant samples. The percentage of mycelial growth inhibition was ranged from 7.08% (KV3) to 84.07% (KV9). Twenty-five isolates showed more than 50% mycelial growth inhibition (Fig. 6). Among the isolates,

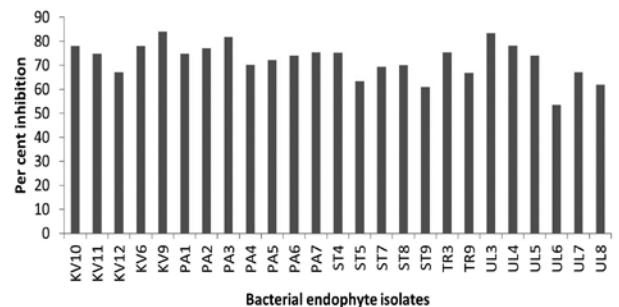


Fig. 6. Percentage inhibition of bacterial endophytes (>50% inhibition) against *P. colocasiae*

Table 4. Mycelial inhibition of *P. colocasiae* by isolated bacterial endophytes

Sl. No.	Isolate code	Tissue used for isolation	Percentage inhibition	Sl. No.	Isolate code	Tissue used for isolation	Percentage inhibition
1	PT1	Root	48.97±0.340	49	PA8	Root	41.3±1.76
2	PT2	Petiole	22.5±0.51	50	PA9	Root	0.0±0.00
3	PT3	Root	0.0±0.00	51	KG1	Root	0.0±0.00
4	PT4	Corm	45.2±1.28	52	KG2	Corm	11.3±1.02
5	PT5	Petiole	47.1±0.32	53	KG3	Corm	47.9±0.29
6	KV1	Petiole	13.95±0.290	54	KG4	Root	34.99±0.510
7	KV2	Root	0.0±0.00	55	KG5	Root	33.5±0.77
8	KV3	Corm	7.08±1.55	56	KG6	Leaf	0.0±0.00
9	KV4	Petiole	26.0±1.65	57	KG7	Petiole	17.5±1.35
10	KV5	Root	0.0±0.00	58	KG8	Leaf	10.3±0.64
11	KV6	Corm	77.95±0.690	59	KG9	Leaf	13.6±0.49
12	KV7	Leaf	44.2±0.23	60	TR1	Root	0.0±0.00
13	KV8	Leaf	28.9±1.36	61	TR2	Corm	39.9±0.15
14	KV9	Petiole	84.1±1.04	62	TR3	Root	75.4±0.52
15	KV10	Petiole	77.95±0.260	63	TR4	Leaf	31.3±2.70
16	KV11	Petiole	74.8±0.26	64	TR5	Root	41.8±0.82
17	KV12	Root	67.0±0.25	65	TR6	Root	0.0±0.00
18	KV13	Root	0.0±0.00	66	TR7	Corm	39.4±0.88
19	IK1	Leaf	12.9±2.06	67	TR8	Corm	0.0±0.00
20	IK2	Root	44.3±0.38	68	TR9	Leaf	66.5±1.14
21	IK3	Leaf	38.1±0.51	69	ST1	Root	32.5±1.34
22	IK4	Corm	24.3±0.64	70	ST2	Corm	0.0±0.00
23	IK5	Petiole	17.3±1.28	71	ST3	Root	28.3±2.12
24	IK6	Root	28.7±1.34	72	ST4	Corm	75.2±0.69
25	MV1	Corm	30.0±1.02	73	ST5	Corm	63.3±0.78
26	MV2	Root	43.6±0.15	74	ST6	Root	0.0±0.00
27	MV3	Petiole	18.5±1.79	75	ST7	Leaf	69.4±0.90
28	MV4	Corm	41.7±1.17	76	ST8	Leaf	69.99±0.450
29	MV5	Corm	0.0±0.00	77	ST9	Leaf	60.9±0.69
30	MV6	Root	0.0±0.00	78	ST10	Petiole	20.95±0.10
31	MV7	Petiole	22.5±1.02	79	ST11	Corm	0.0±0.00
32	MV8	Leaf	20.7±1.97	80	UL1	Root	36.5±0.78
33	MV9	Root	0.0±0.00	81	UL2	Corm	0.0±0.00
34	OT1	Corm	45.8±1.55	82	UL3	Root	83.3±0.90
35	OT2	Root	0.0±0.00	83	UL4	Corm	78.1±0.94
36	OT3	Corm	0.0±0.00	84	UL5	Corm	73.9±1.20
37	OT4	Petiole	25.5±0.41	85	UL6	Petiole	53.5±1.45
38	KM1	Root	19.2±0.58	86	UL7	Leaf	67.0±0.24
39	KM2	Root	26.9±0.51	87	UL8	Leaf	61.9±0.52
40	KM3	Corm	18.5±2.40	88	UL9	Root	0.0±0.00
41	KM4	Corm	0.0±0.00	89	UL10	Petiole	43.3±1.55
42	PA1	Root	74.8±0.26	90	UL11	Leaf	14.8±0.29
43	PA2	Leaf	77.0±0.69	91	TK1	Root	43.3±0.58
44	PA3	Leaf	81.7±0.45	92	TK2	Root	35.1±0.96
45	PA4	Leaf	70.2±0.25	93	TK3	Corm	0.0±0.00
46	PA5	Corm	72.2±0.45	94	TK4	Root	0.0±0.00
47	PA6	Corm	73.9±0.45	95	TK5	Petiole	32.3±1.47
48	PA7	Petiole	75.4±0.52	96	TK6	Corm	0.0±0.00
				97	TK7	Leaf	27.9±0.29

KV9 isolated from a wild taro plant sample showed the highest inhibitory effect of 84.07 ± 1.04 %. A wide range of antagonistic biologically active compounds have been reported to be produced by the endophytic bacteria which may inhibit the growth of fungal pathogens (Jha et al., 2013). In the dual culture, the most effective endophytes which inhibit pathogen growth displayed a significant zone of inhibition. Endophytes isolated from leaves showed the highest number of antagonistic isolates among wild taro plant samples, followed by petiole, corm, and root. In the case of cultivated taro plants, endophytes isolated from roots had the highest number of antagonistic isolates, followed by corm, leaf, and petiole (Fig.7). Even though the number of bacterial endophytes isolated from wild and cultivated taro samples differed significantly, there was no significant variation in antagonistic activity between the isolates from wild and cultivated taro plant samples. The biocontrol activities of rhizobacterial strains from the genera *Bacillus* and *Pseudomonas* against a wide range of plant diseases have been widely researched (Chen et al., 2020; Borriss, 2011; Beneduzi et al., 2012, Kumar et al., 2012).

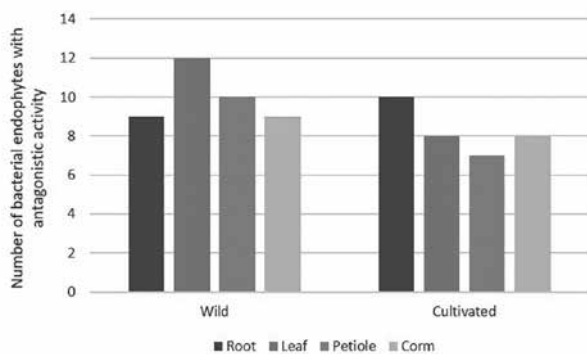


Fig. 7. Distribution of antagonistic bacteria from different taro plant tissues

Conclusion

As concerns about the use of agrochemicals in agriculture grow, the use of bacterial endophytes as biocontrol agents shows tremendous promise for quick adoption to control plant diseases, including *P. colocasiae*, the causal agent of taro leaf blight. Many bacterial endophytes have been identified and tested for antagonistic activity against various plant diseases. However, there was limited information on biocontrol of *P. colocasiae* with biocontrol agents. The current study was successful in identifying 25 most promising endophytic bacteria from taro plants that might be used in integrated disease management. As a result, more research is needed to evaluate the bio control efficacy and plant growth stimulating effects of these promising bacteria before incorporating them into the management of taro leaf blight.

Acknowledgement

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Taro (*Colocasia esculenta* Schott.) based intercropping systems: interspecies interaction effects on growth and yield

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Abstract

Intercropping is a viable option to monoculture with a view to increasing the use efficiency of natural resources. A field experiment was conducted at the Regional Station of ICAR-Central Tuber Crops Research Institute, Bhubaneswar, Odisha, India for three consecutive years (2018-2020) on alfisols under rainfed conditions to study interspecies interaction in taro based intercropping systems. The experiment consisted of seven treatments, T₁-sole taro, T₂-sole maize, T₃-sole pigeon pea, T₄-taro+maize (5:1), T₅-taro+maize (5:2), T₆-taro+pigeon pea (5:1) and T₇-taro+pigeon pea (5:2). The treatments were replicated three times. During the cropping period, the weather was favourable for all the crops in all the years. The results revealed that taro border rows in intercropping resulted in higher growth characters and lower yield components and yield than sole crop rows. Maize and pigeon pea in intercropping resulted in higher growth characters, yield components and yield than sole maize and pigeon pea. Taro was affected by interspecies interference, whereas interspecies interference was minimal for maize and pigeon pea under intercropping. As intercrop, pigeon pea affected taro corm and cormel yield more than maize as pigeon pea competed with taro for longer period (165 days) than maize (90 days). Under intercropping, the decrease in taro corm and cormel yield was due to decrease in taro population apart from intercrop (maize/pigeon pea) competition. Taro corm yield per ha was affected more than cormel yield per ha under intercropping. The cormel equivalent yield (CEY) of taro sole cropping was higher and comparable to taro+maize (5:1) and taro+pigeon pea (5:1) intercropping systems. However, during unfavourable (lesser rainfall and rainy days) season only the potential of intercropping system will be realized.

Keywords: Cormel equivalent yield, Intercropping, Maize, Pigeon pea, Taro

Introduction

Intercropping is a popular production system in small and marginal holdings in developing countries. It allows more efficient use of on-farm resources, provides year-round ground cover or at least for a longer period than monocultures, in order to protect the soil from desiccation and erosion. Growing more than one crop at a time in the same field, enhances water use efficiency

and maintain soil fertility (Rathore, 2016 and Shilpa et al., 2019). Crop insurance is a major principle of intercropping in that if environmental factors change, some of the intercrop does well when others do poorly. Intercropping will not only provide biological insurance against risk of crop failure under aberrant rainfall behaviour in dry land conditions but also ensure more employment opportunities and pest and disease control to some extent (Suja and Nedunchezhiyan, 2018;

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Trupti et al., 2018). In uplands, intercropping and crop substitution stabilize crop yield. When crops of different growth habits are put together in an intercropping system, it provides greater opportunity to secure higher crop yield from the same piece of land than the monoculture largely due to synergetic effect of component crops (Singh et al., 2017). Among component crops, competition is minimal when differences in growth duration are wider in areas having long crop growing period. The components of the crop association need to have different environmental requirements or contrasting habits.

Cereals, millets, pulses, and root and tuber crops are the major food crops grown in rainfed uplands of eastern India. Farmers in this region usually grow more than one food crop in their available landholdings, sometimes, two crops in the same piece of land, separately. Taro (*Colocasia esculenta* Schott.) is grown for its modified stem tubers, which is a rich source of carbohydrate (73-80% on dry weight basis) (Njintang et al., 2007). An annual rainfall of 900-1200 mm spread-over 5-6 months is required for taro cultivation (Nedunchezhiyan and Sahoo, 2019). Taro, a water loving tuber crop is grown in eastern India because of high rainfall and longer crop growing period. However, mid-season and terminal droughts can reduce the yield of taro to a considerable extent. As a sole crop, taro requires huge quantity of seed material (1.2 t ha⁻¹) causing very high initial investment. In small-holder farming system, it may be difficult to invest and during drought, farm economy is severely affected. Hence, intercropping with cereals and pulses under replacement series will reduce seed cost of taro. Intercropping of cereals and pulses in taro can also act as contingent crops and increase the land use efficiency apart from augmenting farm yield in upland rainfed conditions.

Research on growth, yield attributes and yield of component crops in spatially diverse systems such as intercropping can provide an insight on crop competition and cropping pattern design. Investigation carried out in various field experiments revealed that yields of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) were found to increase, whereas soybean (*Glycine max* L.) showed decrease in border rows of strip-intercropping system (Lesoing and Francis, 1999b). In silty clay loam soil, Lesoing and Francis (1999) found that maize yield increased 23% in 8-row alternating strips. In Bhubaneswar (India), Nedunchezhiyan et al., (2011) found that strip intercropping had 10.2 to 44.3% higher sweet potato (*Ipomoea batatas* L.) yield in adjacent border rows than sole crop rows. Sweet potato yields were higher (5.4 to 27.7%) in strip intercropping than in monoculture when calculated across the entire strips in equal area basis (Nedunchezhiyan et al., 2011). The information on yield and yield components of taro and other intercrops when grown in intercropping is not available. If yield components and the spatial cropping

patterns that influence crop yield contributions can be identified, systems can be designed to increase potential productivity. Hence, the present investigation has been carried out to find out the interspecies interaction effects on growth, yield components and yield of taro, maize and pigeon pea (*Cajanus cajan* L.) under intercropping.

Materials and Methods

A field experiment was conducted at the Regional Station of ICAR-Central Tuber Crops Research Institute (20° 14' 50" N and 85° 47' 06" E), Bhubaneswar, Odisha, India for three consecutive years (2018-2000) on alfisols under rainfed conditions. During the crop growing season, the average maximum and minimum temperature were 32.2 and 23.2°C, respectively. The average relative humidity was 74.6%. The total rainfall during crop growing period was 1568.2 mm with 74 rainy days. The climate of the location is characterized by a hot and humid summer, and a cool and dry winter. The soil of the experimental site (top 0.30 m) was having pH 5.7, organic carbon 0.37%, available N 205 kg ha⁻¹, available P 20.1 kg ha⁻¹ and available K 252 kg ha⁻¹. The experiment was laid out in a randomized block design with three replications. The experiment consisted of seven treatments, T₁-sole taro, T₂-sole maize, T₃-sole pigeon pea, T₄-taro+maize (5:1), T₅-taro+maize (5:2), T₆-taro+pigeon pea (5:1) and T₇-taro+pigeon pea (5:2). All the crops in intercropping were planted at 45 × 30 cm spacing. Sole taro at 45 × 30 cm spacing, whereas sole maize and pigeon pea at 60 × 30 cm spacing. The variety Muktakeshi (taro), H-4226 (maize) and CORG 9701 (pigeon pea) were used in this study. The recommended dose of fertilizers N-P-K 80-60-80, 80-40-40 and 20-40-20 kg ha⁻¹ were applied for taro, maize and pigeon pea, respectively. In the intercropping system, the fertilizer dose of respective crops as per net sown area basis was applied. Nitrogen (N), phosphorus (P) and potassium (K) were applied through urea, single super phosphate and muriate of potash, respectively. In all the treatments, half dose of N and full doses of P and K were applied at the time of sowing/planting, while remaining N was applied 1 month after sowing/planting. The experiment was sown/planted during 2nd week of June in all the years. Maize was harvested at 90 days after sowing (DAS), taro was harvested 165 days after planting (DAP) and pigeon pea was harvested 200 DAS.

Observations on growth characters of taro at 90 DAP and, maize and pigeon pea at 90 DAS and maturity were recorded. Observations on yield components and yield of taro, maize and pigeon pea were recorded at harvest. Comparisons were made between border and one middle (inside) row of taro, maize and pigeon pea under intercropping systems. Comparisons between borders and inside rows in a fixed pattern are statistically valid. The cormel equivalent yield (CEY) data was computed taking into the consideration of selling price of taro corm

and cormels, maize and pigeon pea seeds along with their yield.

$$\text{CEY (kg ha}^{-1}\text{)} = \text{Cormel yield (kg ha}^{-1}\text{)} +$$

$$\frac{\text{Corm/maize/pigeon pea yield (kg/ha)} \times \text{Sal price of corm/maize/pigeon pea (.... kg/ha)}}{\text{Sale price of cormel (..... per kg)}}$$

The data were statistically analyzed and significance between mean differences among treatments for various parameters was analyzed using critical differences (CD) at 0.05 probability level.

Results and Discussion

Growth characters of taro

The perusal of data presented in Table 1 revealed that under intercropping, taro growth characters like plant height and number of leaves per hill were higher compared to sole taro at 90 DAP (Table 1). Under intercropping, taro plant height and number of leaves per hill were more in border rows than middle rows (Table 1).

This was due to shade effect caused by the tall intercrop (maize/pigeon pea) grown in taro. The shade effect of intercrop (maize/pigeon pea) was found on taro growth in all the intercropping systems. Two rows of intercrop (maize/pigeon pea) had more effect than one-row on taro plant height and number of leaves per hill (Table 2). Among the intercropping systems, taro plant height and number of leaves per hill were higher in taro+pigeon pea (5:2) than the other intercropping systems (Table 2). This was because pigeon pea offered more shadow due to its branching and a greater number of leaves than maize. Taro shoot dry matter per plant was higher in intercropping than sole cropping at 90 DAP (Table 1). Under intercropping, taro shoot dry matter per plant was more in border rows than middle rows (Table 1). This was due to higher growth characters of taro under intercropping. Two rows of intercrop (maize/pigeon pea) had more effect than one-row on taro shoot dry matter per plant (Table 2). Among intercropping systems, taro shoot dry matter per plant was higher in taro+pigeon

Table 1. Growth characters of taro in various row positions in cropping systems at 90 DAP (Pooled data of 3 years)*

Row position	Plant height (cm)	No. of leaves hill ⁻¹	Shoot dry matter plant ⁻¹ (g)	Corm+cormels dry matter plant ⁻¹ (g)
Taro cropping	74.2±1.0	7.4±0.21	14.9±0.2	58.4±3.6
Tarointercropping				
Border row	94.4±14.0	8.0±0.30	16.1±0.6	44.9±4.1
Middle row	83.5±9.2	7.7±0.29	15.6±0.5	50.8±2.2
Mean row	89.0±11.2	7.9±0.28	15.8±0.5	47.9±3.1

*Mean± Standard deviation

Table 2. Growth characters of taro in intercropping and sole cropping systems at 90 DAP (Pooled data of 3 years)*

Cropping system	Plant height (cm)			No. of leaves per hill			Shoot dry matter plant ⁻¹ (g)			Corm+cormels dry matter plant ⁻¹		
	Border row	Middle row	Mean	Border row	Middle row	Mean	Border row	Middle row	Mean	Border row	Middle row	Mean
Taro	74.9±0.7	73.5±1.2	74.2±0.8	7.5±0.10	7.3±0.31	7.4±0.20	15.0±0.2	14.7±0.3	14.9±0.2	57.0±3.3	59.7±4.5	58.4±3.9
Taro+maize (5:1)	79.8±0.4	75.6±0.5	77.7±0.3	7.7±0.06	7.4±0.20	7.6±0.12	15.3±0.1	15.1±0.1	15.2±0.1	50.4±1.9	53.8±1.5	52.1±1.7
Taro+maize (5:2)	79.8±0.4	75.6±0.5	77.7±0.3	7.7±0.06	7.4±0.20	7.6±0.12	15.3±0.1	15.1±0.1	15.2±0.1	50.4±1.9	53.8±1.5	52.1±1.7
Taro+pigeon pea (5:1)	82.4±1.1	77.1±1.5	79.8±1.3	7.9±0.10	7.6±0.12	7.8±0.10	15.8±0.2	15.3±0.1	15.6±0.1	44.7±1.4	50.4±1.1	47.5±0.6
Taro+pigeon pea (5:2)	106.2±1.6	83.4±1.1	94.8±1.2	8.2±0.06	7.8±0.15	8.0±0.06	16.4±0.2	15.8±0.2	16.1±0.2	44.9±0.3	50.7±0.4	47.8±0.3
Taro+pigeon pea 5:2)	109.3±1.2	97.8±1.5	103.6±0.6	8.4±0.15	8.0±0.23	8.2±0.15	16.7±0.3	16.2±0.4	16.5±0.4	39.7±1.0	48.4±1.3	44.1±0.3

*Mean± Standard deviation

pea (5:2) than the other intercropping systems (Table 2). This was due to greater plant height and number of leaves per hill of taro. However, taro corm and cormel dry matter per plant was higher in sole cropping than intercropping at 90 DAP (Table 1). Under intercropping, taro corm and cormel dry matter per plant was lesser in border rows than middle rows (Table 1). This showed that under shaded conditions the photosynthates present in the shoot could not be translocated to corm and cormels. Two rows of intercrop (maize/pigeon pea) had more effect than one-row on taro corm and cormel dry matter per plant (Table 2). Among intercropping systems, taro corm and cormel dry matter per plant was lower in taro + pigeon pea (5:2) than other intercropping systems (Table 2). This was due to more shade effect inspite of higher shoot dry matter, plant height and number of leaves per hill of taro.

Growth characters of intercrops

Growth characters of maize and pigeon pea were affected by cropping systems. In maize and pigeon pea, greater plant height and number of functional leaves were recorded under intercropping system compared to sole cropping (Table 3) at 90 DAS and harvest. This was mainly due to lesser intra and inter species competition in intercropping than sole cropping. Maize and pigeonpea utilized the available resources efficiently under intercropping. This was also evidenced among intercropping systems. Maize and pigeon pea (border) rows might have a higher relative potential yield advantage owing to greater height difference compared to adjacent taro rows and more competitive advantage in root zone. Nedunchezhiyan (2011) reported similar findings in sweet potato strip intercropping with pigeonpea, maize, rice and ragi. The 5:1 ratio of intercropping recorded higher growth characters than 5:2 ratio. This was due to lesser competition from same species of intercrops in 5:1 than 5:2.

Yield components and yield of intercrops

Yield components of maize and pigeonpea were affected by cropping systems. In maize and pigeon pea, higher number of cobs per pods per plant, number of seeds per cob per pod and 1000 seed weight were recorded under intercropping system compared to sole cropping at harvest (Table 3). This was mainly due to higher growth characters per plant in intercropping than sole cropping (Table 3). The photosynthates stored in shoot was efficiently translocated to developing sink that led to higher yield components. Under intercropping, pigeon pea and maize rows might have a higher relative potential yield advantage owing to greater height difference compared to adjacent taro rows and more competitive advantage in root zone. Among intercropping systems, 5:1 ratio of intercropping resulted in higher yield components than 5:2 ratio (Table 3). This was due to higher growth characters in 5:1 than 5:2. Further, the seed yield per plant was higher in the treatment taro + maize (5:1) compared to the other treatments. This was due to intercropping effect apart from maize genetic character. In all the intercropping treatments, seed yield per plant was higher than sole cropping. Increased number of cobs/pods per plant and seeds/cob or pods might be due to greater light interception by rows in intercropping, resulting in greater photosynthesis rates and development of more cobs/pods and seeds per cob/pod.

The data presented in Table 4 revealed that seed yield (kg ha^{-1}) of maize was higher than pigeon pea irrespective of cropping system. This was due to genetic yield potential of maize. Seed yield (kg ha^{-1}) of maize and pigeon pea was affected by cropping systems. Higher seed yield (kg ha^{-1}) of maize and pigeon pea was noticed in sole cropping compared to intercropping. This was due to higher net sown area under sole cropping than intercropping. Among intercropping systems, 5:2 ratio of intercropping produced higher seed yield than 5:1 ratio

Table 3. Growth and yield components of maize and pigeon pea in intercropping and sole cropping systems (Pooled data of 3 years)

Cropping system	Maize/Pigeon pea							
	At 90 DAS				At harvest			
	Plant height (cm)	No. of functional leaves	Plant height (cm)	No. of functional leaves	No. of cobs plant ⁻¹ or pods plant ⁻¹	No. of seeds cob ⁻¹ or pod ⁻¹	1000 seed weight	Seed yield plant ⁻¹ (g)
Maize	167.2	8.2	164.8	8.1	1.1	194.3	233.1	47.4
Pigeonpea	125.7	135.3	172.8	26.4	158.4	3.8	85.3	25.3
Taro+maize (5:1)	170.1	8.3	169.3	8.3	1.2	231.4	235.5	51.8
Taro+maize (5:2)	168.3	8.2	166.5	8.2	1.2	212.5	234.2	50.3
Taro+pigeon pea (5:1)	130.2	150.2	179.2	30.4	187.2	4.0	8.6	30.7
Taro+pigeon pea (5:2)	127.4	142.8	178.1	28.2	172.9	3.9	86.4	28.1

(Table 4). This was due to the higher net sown area in 5:2 than 5:1. If calculated on the basis of net area sown, seed yield (kg ha^{-1}) of maize and pigeon pea was higher in intercropping than sole cropping (Table 3). Among intercropping systems, 5:1 ratio of intercropping produced higher seed yield than 5:2 ratio (Table 3). This was due to lesser competition within the species as well as higher growth attributes. Nedunchezhiyan (2011) also reported similar findings in sweet potato-based cropping systems.

Yield components and yield of taro

The yield components of taro was influenced by cropping systems. Taro sole cropping produced higher corm and cormel yield per plant both in the border and middle rows compared to intercropping systems (Table 4). In taro sole cropping, both corm and cormel yields were higher in border rows than middle and mean rows (Table 4). The reduction in corm and cormel yield per plant in 5:2 was higher than 5:1 intercropping system. As intercrop, pigeon pea affected taro corm and cormel yield per plant more than maize. This was due to pigeon pea competing with taro for longer period (165 days) than maize (90 days). In intercropping system, corm yield per plant was higher in border rows than middle and mean rows. Whereas, cormel yield per plant was higher in middle and mean rows than border rows. This showed that under shaded conditions, cormel yield was more affected than corm yield with respect to border rows.

Taro corm and cormel yield per ha was found to decrease under intercropping (Table 4). The decrease in taro yield was due to decrease in taro population apart from competition from the intercrop (maize/pigeon pea)

under intercropping. Taro corm yield per ha was more affected than cormel yield per ha under intercropping. The decrease of taro corm yield per ha ranged from 17.1 to 41.9% under intercropping, whereas decrease of taro cormel yield per ha ranged from 16.1 to 38.0% (Table 4). The taro corm and cormel yield per ha was also influenced by intercrops under intercropping. Pigeon pea reduced taro corm and cormel yield per ha more than maize under intercropping (Table 4). This was due to duration of interference of intercrop with main crop. Maize as an intercrop reduced taro corm yield by 17.1-32.9% and cormel yield by 16.1-29.0%, whereas pigeon pea as an intercrop reduced taro corm yield by 26.6-41.9% and cormel yield by 20.7-38% (Table 4). Increasing intercrop population resulted in decrease of taro corm and cormel yield, however it was not linear. When one row of taro was replaced with maize (5:1), the reduction in taro corm and cormel yield was 17.1 and 16.1%, respectively (Table 4). When two rows of taro were replaced with maize (5:2), the reduction in taro corm and cormel yield was 32.9 and 29.0%, respectively (Table 4). Similarly, when one row of taro was replaced with pigeon pea (5:1), the reduction in taro corm and cormel yield was 26.6 and 20.7%, respectively. When two rows of taro were replaced with pigeon pea (5:2), the reduction in taro corm and cormel yield per ha was 41.9 and 38.0%, respectively (Table 4).

The results of CEY revealed that taro sole cropping resulted in higher CEY and it was statistically comparable to taro+maize (5:1) and taro+pigeon pea (5:1) intercropping systems (Table 4). This was due to favourable rainfall during crop growth period of taro and its higher yield. During the three years period of experimentation, the average total rainfall received during the crop growth period was 1568.2 mm with

Table 4. Yield components and yield of taro, and seed yield of intercrops at harvest as influenced by intercropping systems (pooled data of 3 years)

Cropping system	Corm yield plant ⁻¹ (g)			Cormel yield plant ⁻¹ (g)			Corm yield (kg ha ⁻¹)	Cormel yield (kg ha ⁻¹)	Seed yield (kg ha ⁻¹)	Cormel equivalent yield (kg ha ⁻¹)
	Border row	Middle row	Mean	Border row	Middle row	Mean				
Taro	70.2	69.4	69.8	213.3	212.5	212.8	4744	15420	0	18593
Maize	0	0	0	0	0	0	0	0	4862	4862
Pigeon pea	0	0	0	0	0	0	0	0	2101	7006
Taro+maize (5:1)	69.4	64.2	66.8	212.0	212.4	212.2	3934	12934	1344	16900
Taro+maize (5:2)	64.2	62.4	63.3	206.0	210.8	208.4	3184	10945	2122	15187
Taro+pigeon pea (5:1)	59.4	56.8	58.1	198.2	204.2	201.2	3480	12235	627	16647
Taro+pigeon pea (5:2)	55.3	52.3	53.8	178.3	186.5	182.4	2755	9561	892	14373
SEm \pm	-	-	-	-	-	-	-	-	-	667
CD (P=0.05)	-	-	-	-	-	-	-	-	-	2053

*Sale price of corm 10 ₹ kg⁻¹; cormel 15 ₹ kg⁻¹; maize 15 ₹ kg⁻¹; pigeon pea 50 ₹ kg⁻¹

74 rainy days, which was sufficient for raising sole taro crop. During years of lesser rainfall and rainy days, the importance of maize and pigeon pea will be realized. The CEY of taro+maize (5:2) and taro+pigeon pea (5:2) intercropping systems was significantly lower than taro sole cropping. This indicated that if one row of taro was replaced with maize or pigeon pea in an intercropping, they could compensate replaced taro population yield. Thokchom et al., (2016) reported that among taro intercropped treatments maximum taro yield was recorded in combination with single row of cowpea. The reduction in taro yield is compensated by intercrop (cowpea) yield in intercropping. If two rows of taro were replaced with maize or pigeon pea in an intercropping, they could not compensate replaced taro population yield (Table 4). Chhetri and Sinha (2020) also reported that maize+cowpea intercropping system in 2:2 row ratio (replacement series) resulted in higher maize equivalent yield than 2:4 row ratio. The CEY of maize and pigeon pea sole cropping was significantly lowest. This was due to lower seed yield of maize and pigeon pea compared to taro.

Conclusion

It is concluded that growth, yield components and yields of taro, maize and pigeon pea were more affected by intercropping systems. Taro border rows in intercropping showed higher growth characters and lower yield components and yield than sole crop rows. Maize and pigeon pea in intercropping resulted in higher growth characters, yield components and yield than sole maize and pigeon pea. Taro was affected by interspecies interference, whereas interspecies interference was minimal for maize and pigeon pea under intercropping. Hence, they utilized available natural resources more efficiently. As an intercrop, the effect of pigeon pea on taro corm and cormel yield was more than maize because of longer period of competition. The decrease in taro yield was due to decrease in taro population apart from competition from intercrop under intercropping. Taro corm yield per ha was more affected than cormel yield per ha under intercropping. The CEY of taro sole cropping was higher and comparable to taro+maize (5:1) and taro+pigeon pea (5:1) intercropping systems. However, during unfavourable seasons (lesser rainfall and rainy days), especially under the present-day climate change scenario, the potential of intercropping system can be better exploited. To further add to the intercropping advantage, the plant density can be increased in the border rows, use of narrower strips (e.g., alternating two row strips) and growing of intercrops in additive series are recommended. Measurements of light, nutrient, and water use by individual rows across the rows could reveal

more detail on how component crops would be affected. These are the areas for future research.

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Site-specific nutrient management improves soil quality in an ultisol under continuous cassava cultivation

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Abstract

The study aimed to assess the impact of fertilizer applications on soil properties and compute soil quality index (SQI) in a laterite soil under cassava cultivation. The treatments comprised N omission, P omission, K omission, NPK omission, present recommendation (PR) and site-specific nutrient management (SSNM). Soil physico-chemical and biochemical properties were estimated and, selected minimum data set through principal component analysis and soil quality index were developed. Radar diagram was plotted to find out the limiting parameter and correlation between SQI and crop yield was studied. Soil properties such as pH, organic C, labile C, available N, P, K, Ca, Mg, Fe, Mn and Zn showed significant difference among the treatments. SSNM resulted in significantly higher pH (4.60), labile carbon (0.143%), available N (214.82 kg ha⁻¹), Ca (119.70 ppm), Mg (156.15 ppm), Fe (10.20 ppm) and Zn (13.51 ppm) contents. PR treatment showed significantly higher content of organic C (1.17%) and available P (248.44 kg ha⁻¹). Available K and Mn were significantly higher in N omission (472.92 kg ha⁻¹) and NPK (47.80 ppm) omission treatment respectively. Normalised SQI was significantly highest for SSNM (0.86), followed by PR (0.73) and lowest for N omission (0.54), followed by P omission (0.55). No significant correlation was observed between crop yield and SQI. The study indicated that SSNM resulted in improvement of soil quality as revealed from higher SQI.

Keywords: Cassava, site-specific nutrient management, soil quality index, crop yield

Introduction

Cassava (*Manihot esculenta* Crantz), a dicotyledonous perennial shrub belonging to the family Euphorbiaceae and a major food, animal feed and industrial crop of Africa, Asia, and Latin America, grows well in the latitudinal region of 30° north and south of the equator. It grows well in regions where annual rainfall, annual temperature and mean solar radiation is more than 1000 mm, 18°C and 16 MJ m⁻² respectively (Byju et al., 2015). Cassava is well adapted in diverse types of soil and it produces high yields under good crop management in fertile soil. The crop grows well in drained laterite, gravelly and sandy loams soils, while sandy, sandy loam and clay loam are

the soil textural types that favour tuber development and easy harvest (Jose, 2002). For cassava the optimum soil pH is 5.5 and the crop is highly acclimatized to higher acidity ranging from pH 3.7-4.3 (Chew et al., 1981). Cassava is able to yield about 5-6 t ha⁻¹ under poor soil conditions (Cock and Howeler, 1978). At soil temperature ranging from 28-30°C, rapid germination and establishment from stem cutting is noticed, whereas below 17°C or above 37°C sprouting ceases (Keating and Evenson, 1979).

Soil has tremendous capacity to support life through its dynamic functions. It forms the basis of terrestrial life and is an important sink of atmospheric carbon

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dioxide. Unscientific and careless management of soil has resulted in deterioration in quality especially in agricultural lands and industrial areas. So, it is inevitable that the dynamic process of soil management has to be handled with a holistic approach combining the knowledge of all the sciences, depending upon the nature of problems (Verchot et al., 2007). Soil quality in relation to agricultural productivity and sustainability is a growing topic of interest. The fact is that the high yielding varieties and diverse crops intended for increased food production cannot overcome the problems of poor soil quality. So, there is a requirement to develop methodologies that promotes the assessment of soil quality in a region.

There exist different methods of conventional fertilizer management approaches for cassava. Conventional fertilizer management approaches such as blanket recommendations result in lower fertilizer use efficiency, imbalanced NPK applications and thereby deterioration in soil quality (Byju et al., 2016). One of the most promising approaches is site specific nutrient management (SSNM), which is dynamic, and it considers both plant and soil together, the two sides of a coin, and thus both are benefitted. The approach aims at nutrient applications at optimal rates and times for achieving more profits with increased nutrient use efficiency of the crops across time and space; thereby avoiding nutrient loss to the environment. In India, SSNM technology was developed and validated for cassava cultivation based on the modified QUEFTS (Quantitative Evaluation of the Fertility of Tropical Soils) model and was found to increase the yield of cassava by 22 per cent on average (Byju and Suja, 2020).

Materials and Methods

Study site and experimental description

The study site was a 10 year continuous SSNM experimental field in the Research Farm of ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI) Thiruvananthapuram, Kerala, India (8°32'N latitude and 76°55' E longitude, 50 m above MSL). The on-station experiment was started during 2008 and the present study was conducted during 2017-18. The temperature experienced during the crop period (7 months) ranged from 24.03°C to 31.42°C and rainfall 1313.5 mm. The soil in the plot is classed as clayey, skeletal,

isohyperthermic, typic, plinthustults. The average initial nutrient status of SSNM and PR plots showed pH-4.56 and 4.63, OC-1.0 and 1.13%, available N-153.27 and 141.51 kg ha⁻¹, available P-89.18 and 77.89 kg ha⁻¹ and exchangeable K-112.11 and 141.51 kg ha⁻¹ respectively.

The experimental design was a randomised complete block design (RCBD) with six treatments and four replications. A short duration (6 months) variety of cassava, Sree Vijaya, characterised with tuber having very good cooking quality was the test variety. The recorded tuber yield of Sree Vijaya was 25-28 t ha⁻¹, and the starch content was 27-30%. The tuber flesh is yellow colour showing the presence of carotene. The treatments included for the study were nitrogen omission plot (0N), phosphorus omission plot (0P), potassium omission plot (0K), nitrogen, phosphorus and potassium omission plot (0NPK), present recommendation plot (PR) and site-specific nutrient management plot (SSNM). All other crop management practices were done uniformly as per Nair et al., (2004). Details about the soil treatment is shown in Table 1.

Table 1. Details of different soil treatments

Nutrient (kg ha ⁻¹)	Treatment					
	0N	0P	0K	0NPK	PR	SSNM
N	0	150	150	0	100	Customised fertilizer developed for agro ecological unit (AEU) 8 of Kerala, which includes secondary (Ca, Mg) and micronutrients (Fe, Mn, Zn, Cu), (Byju et al., 2016).
P ₂ O ₅	75	0	75	0	50	
K ₂ O	150	150	0	0	100	

Soil sampling and analysis

The soil samples were collected at the time of harvest for estimation of physico-chemical and biochemical properties. From each treatment, representative soil samples were collected. A portion of the sampled soil were air dried and sieved using a 2 mm sieve before various physico-chemical analysis. Remaining portion were used fresh on the same day for soil enzyme studies. The methods adopted for the various soil analysis is shown in the Table 2.

Table 2. Methods used for physico-chemical and enzyme analyses of soil

Parameter	Method	Reference
Soil physical properties		
Single value constants	Keen Raczkowski box method	Wright, 1939
Texture	Hydrometer method	Bouyoucos, 1927
Turbidity ratio	Turbidimetric method	Williams et al., 1966

Soil chemical properties

pH	1:2.5 soil: water suspension, pH meter	Page et al., 1982
Organic carbon	Chromic acid digestion method	Walkley and Black, 1934
Labile carbon	Permanganate method	Weil et al., 2003
Available nitrogen	Micro diffusion method	Janaki and Thyagarajan, 2001
Available phosphorus	Bray and Kurtz No. 1 method, Spectrophotometer	Bray and Kurtz, 1945
Exchangeable potassium	Neutral 1N ammonium acetate, Flame Photometer	Page et al., 1982
Available calcium, magnesium	Neutral 1N ammonium acetate, Atomic absorption spectrophotometer	Page et al., 1982
Available sulfur	0.15% CaCl ₂ , Spectrophotometer	Williams and Steinbergs, 1964
Available Fe, Mn, Zn, Cu	Extraction using 0.1 M HCl, Atomic absorption spectrophotometer	Osiname et al., 1973
Available boron	Extraction using hot water, Azomethine-H method	Gupta, 1967

Soil enzymes

Urease	Colorimetric estimation of urea	Broadbent et al., 1958
Dehydrogenase	TTC assay	Casida et al., 1964
Phosphatase	Colorimetric estimation of p-nitrophenol	Tabataba and Bremner, 1969

Statistical analysis

The experimental data obtained were subjected to the analysis of RCBD and the data interpretation was based on Panse and Sukhatme (1985). At a 5% level of significance, analysis of variance (ANOVA) was used to examine the significance of mean values obtained across treatments. The principal component analysis (PCA) was performed using SSCNARS online portal (<http://sscnars.icar.gov.in>). The soil quality index (SQI) developed by Andrews et al., (2002), which performs very well for small-scale on farm studies was adopted for the study. The basic steps involved in the study were: (i) identification of significant parameters (ii) preparation of minimum data set (MDS) using PCA (iii) normalization of MDS indicators and (iv) indicator scores integration.

Based on results of ANOVA described earlier, all parameters with significant difference among treatments were selected for principal component analysis (PCA). To filter the most suitable indicators for minimum data set (MDS) the data reduction technique PCA was used (Armenise et al., 2013). In the present study, PCA was performed for 11 soil parameters, which were significantly different. The result obtained from the PCA gives a new set of variables 'Principal Components' (PCs). The first principal component (PC) accounts for most of the remaining variability. Eigen value from the PCA depicts the approximate contribution of PC to the total variance (Armenise et al., 2013). Minimization of the indicators was performed on the basis of eigen-one criterion or Kaiser criterion (Kaiser, 1960) and PC

that explains at least 10 per cent of the variance in data were included. According to eigen-one criterion, it is considered that PC1 receiving high eigen values gives best representation of the system and therefore only the PCs with eigen values greater than 1 will be selected.

A weight or factor loading was given to each soil property under certain PC to represent the contribution of the variable to the composition of PC. Only the most highly weighted factors were retained for MDS under each PC. Factor loadings with absolute values less than 10 per cent of the highest factor loading were considered highly weighted (Wander and Bollero, 1999).

To transform the MDS soil properties, linear scoring functions were used, by considering the site-specific characteristics (Table 2). 'More is better function' was used for the all the properties (Liebig et al., 2001; Mukherjee and Lal, 2014). As all soil characteristics of the treatments were below the sufficient level (suggested by ICAR-National Bureau of Soil Survey and Land Use Planning), the score was calculated by dividing each value by the highest value in a particular parameter. All the values of the MDS indicators were transformed to linear functions, where the y-axis ranges from 0 to 1, while the x-axis depicts a site-dependent range (Karlen and Stott, 1994; Andrews and Carroll, 2001). The score 1 was given for highest indicator value. Based on the PCA results, weighted factors were allocated (Table 6). Weights for designated variables were calculated by percent variance in the dataset explained by the selected PCs (Masto et al., 2008). For an indicator in particular PC, full weights was assigned to the indicator.

Table 2. The linear scoring equations used to transform the measured indicator values into scores

Indicator	Equation
Soil pH	$y = 0.2083 \times$
Available N	$y = 0.004 \times$
Available P	$y = 0.0037 \times$
Available Ca	$y = 0.008 \times$
Available Mn	$y = 0.0202 \times$
Available Zn	$y = 0.0614 \times$

The percent variance in the dataset explained by the selected PCs was used to generate weights for selected variables (Masto et al., 2007). Indicator integration into indices was performed using the unscreened transformation using equation (1)

$$\text{Normalized SQI} = \sum_{i=1}^n S_i/n \quad (1)$$

where 'Si' denotes linear scores of observed soil quality indicator, 'n' the number of indicators used in the index.

The final PCA based soil quality equation is

$$\text{SQI} = \sum_{i=1}^n W_i/S_i \quad (2)$$

Where 'W' is the PC weighting factor and 'S' is the indicator score.

The normalized SQI was finally calculated to limit the SQI values in the range 0 to 1. The higher the index score, the better the soil quality or the better the soil function. For each treatment, the per cent contribution of each selected indicator to the total SQI was determined. Limiting soil parameter among the selected indicators was identified using radar diagram and the effect of treatments in crop yield was also assessed. The overall step in the process of development of soil quality index is shown in Fig. 1.

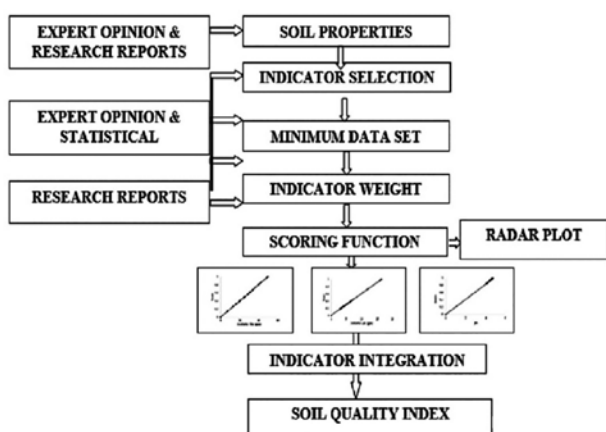


Fig. 1. Steps involved in development of soil quality index (SQI) (modified after Masto et al., 2007)

Results and Discussion

Soil physico-chemical properties and enzyme activities

The effects of different treatments on soil physical properties are presented in Table 3. The physical properties did not show any significant difference among the six treatments. Though not significant, highest bulk density was recorded in N omission treatment (1.29 Mg m^{-3}) and lowest for present recommendation (PR) (1.22 Mg m^{-3}), closely followed by site specific nutrient management (SSNM) (1.23 Mg m^{-3}). A slight improvement in water holding capacity was recorded in SSNM (40.99%) and PR (40.84%) treatments compared to nutrient omission treatments. Highest values of porosity (55.82%) and turbidity ratio (0.46%) were recorded in SSNM treatment.

Stockdale et al., (2001) reported that it took decades to establish quantifiable changes in soil physical properties. Slight changes in soil physical properties under organic farming was reported by Suja et al., (2012). Similar results was also reported by Madhavi et al., (2020) in cassava cultivated soils.

The effect of different treatments on soil chemical properties is shown in Table 4. A significantly higher pH was observed in SSNM treatment (4.60), which was on par with PR (4.57), N omission (4.55) and K omission (4.46) treatments. Significantly lower pH was observed in P omission treatment (4.14). The organic carbon was significantly higher for PR (1.17%) treatment, followed by SSNM (1.15%) and P omission (1.06%) treatment. Significantly lower OC content was recorded in K omission and NPK omission treatments (0.82%). Significantly higher labile carbon content was found in SSNM treatment (0.14349), which was on par with P omission (0.14347%) and PR treatments (0.14346%), while significantly lower labile carbon was recorded in N omission (0.14333%), followed by NPK omission (0.14335%) treatments. Significantly higher available N content was observed in SSNM treatment ($214.82 \text{ kg ha}^{-1}$), which was on par with PR treatment ($192.08 \text{ kg ha}^{-1}$). Significantly lowest available N content was observed in N omission treatment ($126.22 \text{ kg ha}^{-1}$), followed by NPK omission treatment ($133.67 \text{ kg ha}^{-1}$). Available P content was significantly higher in PR treatment ($248.44 \text{ kg ha}^{-1}$), followed by SSNM ($242.47 \text{ kg ha}^{-1}$) and was significantly lower in N omission ($160.62 \text{ kg ha}^{-1}$), P omission ($169.61 \text{ kg ha}^{-1}$) and K omission ($105.06 \text{ kg ha}^{-1}$) treatments. Available K content was significantly higher in N omission treatment ($472.92 \text{ kg ha}^{-1}$), while it was significantly lower in K omission treatment ($105.06 \text{ kg ha}^{-1}$), followed by NPK omission treatment ($148.90 \text{ kg ha}^{-1}$).

Among the secondary nutrients, Ca and Mg showed significant difference among the treatments. A

Table 3. Effect of treatments on physical properties of soil

Treatments	Sand	Silt	Clay	BD*	WHC*	Porosity	TR*
	%	%	%	Mg m ⁻³	%	%	-
N omission	73.93	1.60	24.48	1.29	39.83	54.51	0.14
P omission	73.55	1.85	24.60	1.26	39.56	52.41	0.18
K omission	73.55	1.78	24.67	1.27	38.48	52.83	0.13
NPK omission	73.93	1.38	24.70	1.24	39.97	51.52	0.35
PR	74.05	1.73	24.23	1.22	40.84	52.63	0.26
SSNM	73.92	1.78	24.30	1.23	40.99	55.82	0.46
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS

*BD- bulk density (Mg m⁻³), WHC- water holding capacity in (%), TR- turbidity ratio

Table 4. Effect of treatments on chemical properties of soil

Treatments	pH	OC*	LC*	Available N	Available P	Exchange-able K	Available Ca	Available Mg	Available S	Available Fe	Available Mn	Available Zn	Available Cu	Available B
		%	%	N kg ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹
ON	4.55	0.86	0.14333	126.22	160.62	472.92	88.80	33.80	15.19	7.50	22.75	3.66	1.55	0.97
OP	4.14	1.06	0.14347	146.61	164.71	286.83	67.20	22.33	8.91	8.62	33.62	4.59	1.79	0.80
OK	4.46	0.82	0.14338	163.07	169.91	105.06	92.30	29.75	14.49	6.80	21.76	4.55	1.62	0.66
ONPK	4.35	0.82	0.14335	133.67	199.22	148.90	90.40	40.60	19.84	8.48	47.80	4.31	1.78	0.70
PR	4.57	1.17	0.14346	192.08	248.44	387.74	111.40	99.00	20.08	10.06	21.38	6.74	1.73	0.72
SSNM	4.60	1.15	0.14349	214.82	242.47	395.02	119.70	156.15	18.21	10.20	30.22	13.51	1.90	1.26
CD (0.05)	0.24	0.215	0.0001	45.522	26.15	74.92	15.20	68.771	NS	1.41	12.832	2.04	NS	NS

*OC - organic carbon, LC - labile carbon

significantly higher available Ca content was observed in SSNM treatment (119.7 ppm), followed by PR treatment (111.4 ppm), while significantly lower Ca content was recorded in P omission treatment (67.20 ppm). The SSNM treatment showed significantly higher Mg content (156.15 ppm) and P omission showed significantly lower Mg (22.33 ppm), which was on par with K omission treatment (29.75 ppm). Among the micronutrients, Fe, Mn and Zn showed significant variation among the treatments. A significantly higher available Fe was observed in SSNM treatment (10.20 ppm), which was on par with PR (10.06 ppm) and significantly lower Fe content in K omission treatment (6.80 ppm). Available Mn was significantly higher in NPK omission (47.80 ppm) while it was significantly lower in PR (21.38 ppm), which was on par with N (22.75 ppm), P (33.62 ppm), K (21.76 ppm) omission and SSNM (21.38 ppm) treatments. The SSNM treatment (13.51 ppm) showed significantly higher available Zn, while N omission treatment showed significantly lower values and was on par with P (4.59 ppm), K (4.55 ppm) and NPK (4.31 ppm) omission treatments.

Apart from NPK, addition of farmyard manure and litter fall in SSNM and PR plots have contributed to significant increase in the OC and NPK content. Further in SSNM treatment secondary nutrients such as Ca and Mg, and micronutrients Fe, Mn, Zn and Cu were applied along with customized formulation. This is the reason for significant increase in these nutrients in SSNM treated soil than others. Similar result was reported by Madahavi et al., (2020).

The results of soil enzyme activities are presented in Table 5. No significant differences in soil enzyme activities could be observed. The value of urease activity was highest in SSNM (1473.31 $\mu\text{g g}^{-1} \text{h}^{-1}$) and lowest in K omission (1138.12 $\mu\text{g g}^{-1} \text{h}^{-1}$). The value of phosphatase was highest in PR (302.21 $\mu\text{g PNP g}^{-1} \text{h}^{-1}$) and lowest in P omission (203.69 $\mu\text{g PNP g}^{-1} \text{h}^{-1}$). The highest and lowest value of dehydrogenase were observed in NPK omission treatment (27.85 $\mu\text{g TPF g}^{-1} \text{h}^{-1}$) and SSNM treatment respectively. As there are no organic treatments no significant increase in enzyme activity was noticed in the soil of any treatments.

Table 5. Effects of treatments on soil enzyme activities

Treatments	Urease $\mu\text{g g}^{-1} \text{h}^{-1}$	Dehydrogenase $\mu\text{g TPF g}^{-1} \text{h}^{-1}$	Phosphatase $\mu\text{g PNP g}^{-1} \text{h}^{-1}$
N omission	1288.55	21.22	248.72
P omission	1139.42	20.79	203.69
K omission	1138.12	18.47	244.49
NPK omission	1177.69	27.85	251.14
SSNM	1473.31	15.98	259.90
PR	1359.81	27.59	302.21
LSD (0.05)	NS	NS	NS

Principal component analysis (PCA)

Soil quality index (SQI) was computed to evaluate the effect of different treatments on soil quality. For the principal component analysis (PCA), the soil parameters that showed significant difference among the treatments were selected. By taking into account the aforementioned soil characteristics, 11 PCs were generated using the principal component analysis (Table 6).

Table 6. Results of principal component analysis of soil quality indicators for the first three PCs selected for computing SQI

Principal Components	PC 1	PC 2	PC 3
Eigen value	5.15	1.55	1.40
Loading factor	0.64	0.19	0.17
Per cent	46.84	14.11	12.72
Cumulative percent	46.84	60.95	73.66
Eigen vectors			
pH	0.219	-0.562	0.342
OC	0.300	0.201	-0.397
LC	0.243	0.183	-0.569
N	0.373	-0.079	-0.926
P	0.364	0.193	0.248
K	0.211	-0.336	-0.161
Ca	0.346	-0.016	0.359
Mg	0.327	0.183	0.184
Fe	0.335	0.128	-0.008
Mn	-0.644	0.635	0.378
Zn	0.380	-0.022	0.009

With reference to Kasier (1960) criterion, the number of datasets that can be included was limited up to PC3, as from PC4 onwards the eigen value dropped below 1. The three PCs together contributed 73.67%

of the total variance, while the residual components only marginal. The percentage explained by PC1, PC2 and PC3 are 46.84, 14.11 and 12.72% respectively of the total variance. In PC1, Mn showed very high value, which is double than that of other eigen vectors and so it was considered in PC2. Thus in PC1, Zn, P and N were the highest weighed variables, while for PC2 they were Mn and soil pH. Though N showed very highly weighed variable in PC3 it was not considered as it was already considered in PC1. Considering the rest of eigen values, LC showed highest value but the critical difference among the treatments was very low and so it was not considered. As Mn was considered in PC2 it was discarded. Thus, in PC3 highest weighed variable considered was Ca. Therefore, the final dataset contains only 6 variables, namely pH, N, P, Ca, Mn and Zn.

Soil quality index (SQI)

The final equation for normalised PCA based soil quality is:

$$\text{Normalised SQI} = 0.636 [(SN+SP+SZn)/3] + 0.192 [(SpH+SMn)/2] + 0.173 (SCa/1)$$

Fig. 2 and Table 7 shows normalised cumulative soil quality indices for the various treatments.

The contribution of the MDS indicators (scored and weighed) to the overall index value is represented by the bars.

Table 7. Normalised cumulative soil quality indices for different treatments

Treatments	Normalised SQI
N omission	0.54
P omission	0.55
K omission	0.59
NPK omission	0.63
PR	0.73
SSNM	0.86
LSD	0.094

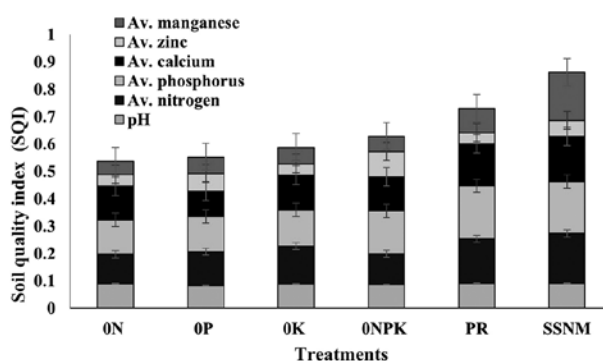


Fig. 2. Effect of different fertilizer treatments on soil quality index (SQI)

Significant differences between the treatments were showed in the normalised cumulative SQI values by the various treatments. The SSNM treatment (0.86) showed significantly higher soil quality index, which was followed by PR treatment (0.73) and significantly lower SQI was shown by N omission treatment (0.54), followed by P omission treatment (0.55). The soil parameters that have contributed to the increase in SQI in SSNM are available N, P, Ca and Mn. Madhavi et al., (2020) has reported an increase in SQI in SSNM treated soil under cassava.

Correlation of soil quality index and tuber yield

The yield of a crop depends on environmental, biological and technological factors. Improvement in SQI contributes to the availability of essential nutrients to the crop. But in this study no linear correlation was found between crop yield and SQI indicating that the factors that are not considered in the study have also contributed to the tuber yield (Fig. 3). This result is in tune with Armenise et al., (2013), which states that ‘these were either not soil related or due to ‘patchy’ spatial variation of soil quality in the field’.

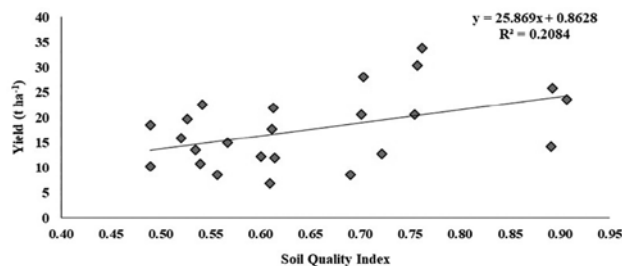


Fig. 3. Correlation of soil quality index (SQI) values and tuber yield

Conclusion

The study has contributed for evaluating the impact of different treatments on soil quality. The study indicates that application of SSNM for ten years has not imparted a significant effect on physical and enzyme activities of the soil, but on chemical parameters. No correlation was noticed between SQI and tuber yield, indicating that the indicators selected for the SQI are not the only factors that contributes to yield but is a combination of various climatic and edaphic factors. Thus, it can be concluded that continuous application of SSNM for 10 yrs has improved soil quality.

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Molecular identification of tortoise beetle and its endosymbiotic bacteria

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Abstract

Tortoise beetles are one of the important defoliator pests of sweet potato, 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 aphids will help to develop important strategies for the management of this noxious pest. Cassidini is the largest tribe of tortoise beetle in Kerala represented by 18 species in four genera, followed by Aspidimorphina and notosacanthini. In the present study, molecular characterization of the sweet potato defoliator and endosymbiotic bacteria associated with them, was done. By molecular characterization they were identified as *Chiridopsis* sp. and sequences were deposited at NCBI with accession no OR416859. Morphological characters of the isolated revealed that each isolate has different colony characters. 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 *Kosakonia cowanii* and *Kosakonia* sp. The 16S rRNA gene sequences were also deposited at NCBI database with accession no OR426444, OR418414. From the aligned sequences phylogenetic tree was constructed by the Neighbor- Joining method using MEGA version 11.

Keywords: Sweet potato, tortoise beetle, 16S rRNA, endosymbiotic bacteria

Introduction

The symbiotic microorganisms attached with the herbivorous insects always play major role in lifecycle of insects. In particular, these microorganisms can supplement essential amino acids or vitamins, or enzymes for digestion or detoxification of noxious plant secondary metabolites in their host diet (Douglas, 2009; Douglas, 2015; Feldhaar, 2011). Hence, these endosymbiotic associations with beneficial microbes always provide essential nutrients to the hosts. Many insects such as wood-feeding termites, passalid beetles and the leaf-chewing tortoise beetles are assisted by symbiotic

microbes for the break-down of the major components of plant cell walls that are enzymatically challenging sources of carbon and energy (Brune, 2014; Navarro, et al., 2019; Salem, 2017; Cortes et al., 2012). Tortoise beetles (subfamily Cassidinae) are one of the specialized herbivores that feed on sweet potato leaves (McKenna., 2020). These are associated with a broad range of host plants. Many of the species *Aspidimorpha*, *Chiridopsis*, *Lacoptera* and *Cassida* infest sweet potato plants.

Recent genetic analysis studies revealed that the thistle tortoise beetle lacks the genes responsible for the production of the pectinases. These studies also revealed

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that the pectin present in leafy plant parts eaten by the beetle digested by symbiotic γ -proteobacterial symbiont, *Stammera*. These leaf beetles are able to degrade components of the plant cell wall, such as cellulose and pectin, with the help of digestive enzymes by these bacteria. Moreover, these bacteria possess reduced genome and possess pectinases enabling the host to digest the foliage. The tortoise beetle genome does not encode pectin digesting enzymes but they obtain nutrients with the help of these bacteria. These bacteria reside in sac-like organs in the adult and larval tortoise beetle foregut, where they produce pectinases. They also reside in the reproductive tract of adult females, where they play a role in bacterial transmission to beetle offspring (Salem et al., 2020). Their genome also lacks many genes that code for essential cellular functions that are typically found in free-living bacteria which made them completely dependent on the beetle (Salem et al., 2017). In darkling beetles of the subfamily Lagriinae (Tenebrionidae), specific β -proteobacteria of the genus *Burkholderia* are associated with larvae and adult females extracellularly and provide protection against pathogens by producing antibiotics (Florez et al., 2017; Kaltenpoth and Florez, 2020). Therefore, the knowledge about interaction between insects and symbionts is gaining importance in agriculture due to the potential application for the management of insect pests. Some of the insect gut symbionts are capable of enhancing insecticide resistance in several insect species (Kikuchi 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). Hence the complete exclusion of these primary endosymbionts from insects may reduce their lifespan and suppress population within a few days or weeks.

Previous studies on tortoise beetles of Kerala reported that *Aspidimorpha furcata*, *Aspidimorpha miliaris*, *Aspidimorpha sanctaecrucis*, *Cassida circumdata*, *Chiridopsis bipunctata*, *Lacoptera nepalensis*, *Aspidimorpha fuscopunctata* infest sweet potato plants. It is also reported that the tortoise beetles infest about 25 species of plants belonging to the family Convolvulaceae. (Takizawa, 1980; Ghate et al., 2003; Borowiec and Swietojanska, 2020; Amritha Hari 2020).

Materials and Methods

Collection of insects

Adult tortoise beetles were collected and maintained at the Entomology Laboratory, Division of Crop Protection, ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram.

DNA isolation from insect

The genomic DNA was isolated using the modified cetyltrimethyl ammonium bromide buffer (CTAB) method

Gawel and Jarrett (1991). The individual insect samples were homogenized with 500 μ l of lysis buffer (CTAB 2%, 100 mM Tris-HCl (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 h 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 microcentrifuge tube and DNA was precipitated by adding 40 μ l of sodium acetate, 600 μ l of 95% ethyl alcohol. The tubes were kept at -20°C 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 μ l 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 μ L⁻¹.

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 10 min, 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). PCR was performed in 25 μ L total reaction volume containing 20 Pico moles of each primer, 1.0 μ L of 20 mM dNTP, 2.5 μ L of 10X buffer and 1.0 μ L 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 ng μ L⁻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 beetles were collected and 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

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 (Sreerag et al., 2014)

Phenotypic characterization of ESB strains

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 100× 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

PCR amplification of 16S rDNA gene by universal primers: forward primer fD1 5'AGAGTTTGATCCTG GCTCAG3' and reverse primer RP2 5'CGGCTACCTT GTTACGACTT3' (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 MgCl₂, 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 visualised under UV transilluminator and the purified PCR products of 1500 bp were sequenced at Eurofins Genomics India Pvt. Ltd., Bengaluru, Karnataka.

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 sweet potato defoliator

The tortoise beetles collected from one month old sweet potato plants maintained at ICAR-CTCRI. The molecular identification of insects was done. 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 phylogenetic 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 *Chiridopsis* sp. isolate TB

Phenotypic and molecular characterization of bacterial isolates

A total of two isolates were isolated from the *Chiridopsis* sp. and were assigned code numbers as isolates T1 and T2. Morphological variations were observed for each endosymbiotic bacterial strain, but no pigmentation was observed. Colonies formed on nutrient agar were circular, raised, convex, flat, entire white in colour with no pigmentation. A total of two bacterial strains were successfully isolated from the *Chiridopsis* sp. and were assigned code numbers as isolates T1 and T2 for laboratory purposes. They were gram positive, rod-shaped bacteria. 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 isolates T1 and T2 showed 98 % similarity to *Kosakonia*

cowanii and *Kosakonia* 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 phylogenetic tree of the endosymbiotic bacteria based on 16S rRNA gene sequences is shown in (Fig. 2).



Fig. 2. Phylogenetic tree inferred from 16S rRNA gene sequences analysis of bacterial isolate T1 and T2 associated with *Chiridopsis* sp.

Table 1. Molecular identification of tortoise beetle and endosymbiotic bacteria

Isolate	Identification	Accession No.	Similarity (%)
T1	16S ribosomal RNA gene sequence partial <i>Kosakonia cowanii</i> isolate T1	OR418414	84
T2	16S ribosomal RNA gene sequence partial <i>Kosakonia</i> sp. isolate T2	OR426444	84
TB	Mitochondrial cytochrome c oxidase subunit I (COX1) gene sequence partial <i>Chiridopsis</i> sp. isolate TB	OR416859	97

Previous studies have reported specific gut-inhabiting yeast-like symbiotic fungi from cigarette and drugstore beetles which provide sterols to their hosts (Pant and Fraenkel, 1954; Noda and Koizumi, 2003). Similarly in leaf beetles belonging to the subfamilies Donaciinae (Chrysomelidae), specific gut-inhabiting γ -proteobacteria, *Macrolepicola* supply enzymes for plant digestion and essential nutrients for their hosts (Reis et al., 2020). *Kosakonia* has been reported to be associated with plant growth promoting bacteria (Kampfer et al., 2005; Peng et al., 2009). In our study *Kosakonia cowanii* was found to be reported with the *Chiridopsis* sp. and *K. cowanii* was identified as the causal agent of bacterial

wilt on tomatoes (Sarkar and Chaudhuri, 2015). The genus *Kosakonia* is recently derived from reclassification of genus *Enterobacter*, and several species, including *Enterobacter arachidis*, *Enterobacter cowanii*, *Enterobacter oryzae*, *Enterobacter radicincitans* previously included in genus *Enterobacter* which have been transferred to the novel genus *Kosakonia* (Brady et al., 2013). *K. cowanii* is reported as phytopathogen causing a variety of plant diseases viz pathogen of eucalyptus and woody plants (Furtado et al., 2012; Wu et al., 2016; Krawczyk and Borodynko-Filas, 2020). Some strains of this species are also opportunistic human pathogens (Grimont and Grimont, 2006; Mardaneh and Soltan-Dallal, 2014; Peleg et al., 2008; Yang et al., 2018). The ability of the species to colonize different ecological conditions revealed its easy adaptable metabolism to new host conditions. Earlier report also showed the ability to cause necrotic spots on leaves of *Mabea fistulifera* Mart. (Euphorbiaceae) (Furtado et al., 2012). Some of the reports also showed *K. cowanii* colonizing the gut of *Anopheles gambiae* displayed direct anti-Plasmodium properties (Dennison et al., 2014). Previous studies conducted using *K. cowanii* B-6-1 isolated from tomato showed biocontrol activity against *E. verticillioides*, *A. tenuissima*, and *B. cinerea* (Shi and Sun, 2017). *K. cowanii* was found in microbiome of wasp and as an endophytic growth-promoting bacteria in some plants (Meng et al., 2015, Fall and Holley, 2016).

Conclusion

The endosymbiotic bacteria exist within the insect host and play major role in the metabolic process of the insects. They serve as unexploited sources of enzymes, bioactive molecules which helps the insect host to survive in extreme environmental conditions. Currently the research in insect-symbiosis using various genomic tools had led to the identification of the genes which are responsible for the production of these enzymes as well as various bioactive molecules which will be useful for various insect pest management strategies.

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- comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis*, *E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter zurichensis* nom. nov., *Cronobacter helveticus* comb. nov. and *Cronobacter pulveris* comb. nov., respectively, and emended description of the genera *Enterobacter* and *Cronobacter*. *Syst. Appl. Microbiol.*, **36**:309–319.
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Optimisation of Callus Induction in the Leaf and Stem Tissues of the Orange Flesh Sweet potato Variety Bhu Sona

Sweet potato (*Ipomoea batatas* (L.) Lam), belonging to the family Convolvulaceae, is one of the important food crops cultivated globally ~7.4 million hectares, with a total production of ~92 million tons (FAOSTAT, 2019). It is cultivated throughout the tropical and subtropical regions of the world, including India (Edison et al., 2009; Tava and Nedunchezhiyan, 2012). Because of its short growing period, ability to grow in diverse environmental conditions and high yield potential, sweet potato is considered as one of the potential crops that can help us to meet the future energy and nutritional needs of both human and livestock populations particularly in developing countries (Motsa et al., 2015; de Albuquerque et al., 2019). The introduction of β -carotene rich orange-fleshed sweet potato varieties in dietary programs has alleviated vitamin-A deficiency among children and pregnant women in many developing countries (Girard et al., 2017; Govender et al., 2019). In India, ICAR-CTCRI and different AICRP-TC centres have released six β -carotene rich varieties viz., Bhu Sona, Bhu Kanti, BhuJa, Gouri, Kamala Sundari and Co-5 (Sunitha et al., 2018). Development of *in vitro* plant regeneration from various plant tissues is important for various basic biotechnology applications such as virus elimination, germplasm conservation, including plant propagation and genetic improvement (Arathi et al., 2019; Ravi et al., 2020; Lenka et al., 2018). In tissue culture, plant hormones influence the differentiation and growth of the explants (Hill and Schaller, 2013). In addition to these, several factors, including genetic factors/genotype differences, the type of explants used for regeneration, the selection of hormones and the concentration used, are also known to determine the regeneration efficiency of the plants (Salari et al., 2013).

Several studies have shown that the callus induction and regeneration efficiency in many crop plants, including sweet potato is highly genotype dependent (Ravi and Indira, 1999; Salari et al., 2013; Arathi et al., 2019). Due to these differential responses of the cultivars, genotype specific callus induction protocol needs to be developed for *in vitro* culture based genetic improvement studies (Salari et al., 2013; Arathi et al., 2019). Moreover, development of callus induction protocols would be helpful in the improvement of important traits through

biotechnological approaches (Muthusamy et al., 2017; Lenka et al., 2019; Jagannadham et al., 2021). Arathi et al., (2019) used the growth regulators viz., BAP, TDZ, GA₃ and IBA and found that the MS media supplemented with 0.5 mg l⁻¹ BAP and 1.0 mg l⁻¹ GA₃ displayed good shooting efficiency in sweet potato. Several workers had shown that Naphthalene Acetic Acid (NAA), a synthetic auxin hormone has good effect on callus induction in many crops (Ahmad and Spoor, 1999; Nazir et al., 2020). The effect of NAA on induction of callusing is yet to be studied in the orange-fleshed sweet potato Bhu Sona. Hence, in the present study, the NAA hormone was used for optimisation of callus induction in leaf, stems and root tissues of the Bhu Sona variety.

The study was conducted in the ICAR-Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, during the period 2020-2021. Bhu Sona, sweet potato variety of ICAR-CTCRI, was used in this study to optimise callus induction. Microbial contamination emanating from both endogenous and exogenous sources remains one of the serious concerns in the *in vitro* culture (Amisshah et al., 2016). To reduce microbial contamination from the outside environment, first, we initiated the *in vitro* cultures for multiplication of the variety Bhu Sona. The four weeks old plantlets were cut into a smaller size of 3-4 cm shoots and multiplied in the sterile tubes containing MS media with 3% sucrose and 0.8% charcoal. The plantlets were grown in controlled conditions at 25±2°C temperature under a photoperiod of 16 h light and 8-h dark in the plant tissue culture room. Contamination free, four weeks old grown plantlets with uniform size were selected to obtain explants required for callus induction study (Fig. 1).



Fig. 1. Initiation of contamination free fresh *in vitro* plantlets of Bhu Sona.

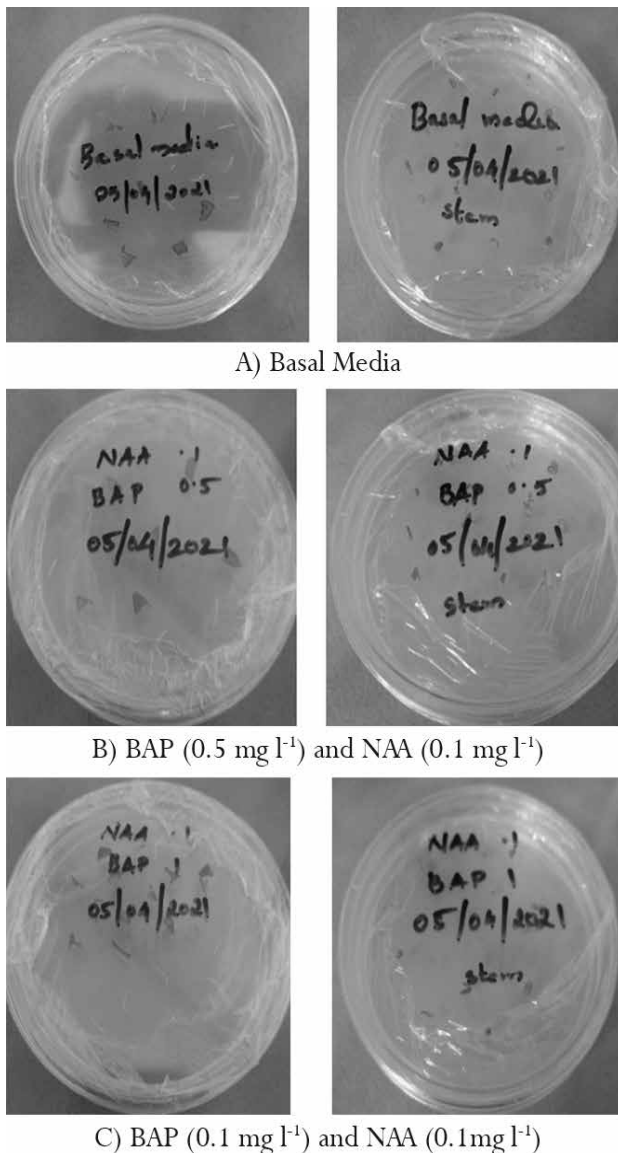


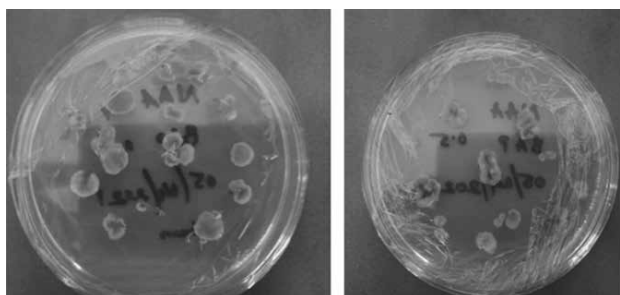
Fig. 2. Culturing of leaf, stem and root tissues of sweet potato for induction of callusing in MS media with different hormone combinations.

The callus induction potential of the variety Bhu Sona was studied in different hormonal combinations viz., Basal media (MS media supplemented with 3% sucrose); basal media supplemented with 0.1 mg l⁻¹ NAA+0.1 mg l⁻¹ BAP and basal media supplemented with 0.1 mg l⁻¹ NAA+0.5 mg l⁻¹ BAP (Table. 1 and Fig. 2). Leaf, stem and root segments, about (0.3 to 0.8 cm) long were excised from four weeks-old *in vitro* raised plants and cultured in the petri dishes containing three media combinations viz., Basal media (MS media with 3% sucrose), basal media supplemented with 0.1 mg l⁻¹ NAA+0.1 mg l⁻¹ BAP and basal media supplemented with 0.1 mg l⁻¹ NAA+0.5 mg l⁻¹ BAP were used to study the effects of plant growth regulators on callus induction from leaf, stem and root tissue of sweet potato. For evaluating callusing efficiency of the leaf tissues 14, 16, and 14 leaf explants were placed in basal media, 0.1 mg l⁻¹ NAA+0.5 mg l⁻¹ BAP and 0.1 mg l⁻¹ NAA+0.1 mg l⁻¹ BAP MS plates, respectively whereas, for evaluating stem tissues 18, 13 and 12 stem explants were placed in basal media, 0.1 mg l⁻¹ NAA+0.5 mg l⁻¹ BAP and 0.1 mg l⁻¹ NAA+0.1 mg l⁻¹ BAP MS plates, respectively (Table 1 and Fig. 2) and for evaluating root tissue, 8, 6 and 8 root explants were placed in 0.1 mg l⁻¹ NAA 0.5 mg l⁻¹ BAP and 0.1 mg l⁻¹ NAA+0.1 mg l⁻¹ BAP MS plates, respectively (Table 1 and Fig. 2). The petri plates were then sealed with parafilm and cultures were incubated in a culture room at 25±2°C temperature under a dark condition. Additionally, charcoal (0.8%) was included in the growth media to act as an adsorbent of phenolic compounds of the cultures (Arathi et al., 2019).

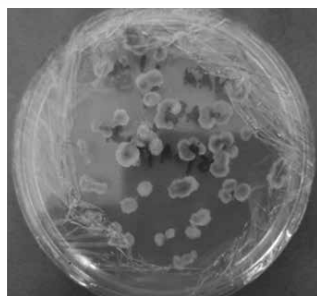
The plates were incubated in dark conditions with a room temperature of 25±2°C in the tissue culture room. One hundred percent callus induction efficiency was observed in both leaf and stem tissues on the MS media supplemented with the hormones NAA and BAP, whereas, in basal media lacking these hormones failed to show the callus induction (Table 1 and Fig. 3). However, the root tissues failed to show callus induction in all three

Table 1. Callus induction in leaf, stem and root tissues of sweet potato under different hormone treatments

Explant	Media Composition	No of Explants inoculated	Callus induced
Leaf	Basal media	14	0
	MS+0.1 mg l ⁻¹ NAA+0.5 mg l ⁻¹ BAP	16	16
	MS+0.1 mg l ⁻¹ NAA+1 mg l ⁻¹ BAP	14	14
Stem	Basal media	18	0
	MS+0.1 mg l ⁻¹ NAA+0.5 mg l ⁻¹ BAP	13	13
	MS+0.1 mg l ⁻¹ NAA+1 mg l ⁻¹ BAP	12	12
Root	Basal media	8	0
	MS+0.1 mg l ⁻¹ NAA+0.5 mg l ⁻¹ BAP	6	0
	MS+0.1 mg l ⁻¹ NAA+1 mg l ⁻¹ BAP	8	0



A) BAP (0.5 mg l^{-1}) and NAA (0.1 mg l^{-1})
(leaf and stem tissues)



B) BAP (0.1 mg l^{-1}) and
(0.1 mg l^{-1}) (leaf and
stem tissues)

Fig. 3. Callusing in leaf, stem and root tissues
of sweet potato

media compositions (Table 1 and Fig. 3). A combination of NAA and BAP has shown success in the induction of callusing in many plants (Ahmad and Spoor, 1999; Nazir et al., 2020). Arathi et al. (2019) have used the growth regulators viz., BAP and IBA for induction of the callusing in sweet potato varieties Gowri and Bhu Sona, however, the callus developed from the leaf and stem tissues failed in organogenesis. Hence, in this study we have used the growth regulators viz., BAP and NAA for callus induction. However, the callus developed from the leaf and stem tissues were failed in organogenesis. Thus, further studies with different hormone combinations, including statistical parameters would be helpful in the standardisation of robust protocol for callus induction and regeneration of the explants in sweet potato.

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A New Nutrient Rich Biofortified Greater Yam Variety: Gujarat Greater Yam-1 (Hemlata)

Biofortified greater yam assumes an important role in achieving food security and prevent malnutrition and hunger issue in the population. About 800 million people suffer from hunger, but even more suffer from micronutrient malnutrition, also called “hidden hunger”. Among these, iodine, vitamin A, iron and zinc malnutrition are the major concerns. Biofortification provides a comparatively cost-effective, sustainable and long-term means of delivering more micronutrients in relatively remote rural areas and it also delivers naturally-fortified foods to population groups with limited access to commercially-marketed fortified foods (Gomathi et al., 2017). Root and tuber crops, including cassava, sweet potato, greater yams, potato, cocoyam and other minor tuber crops are important to the agriculture and food security of many countries and are the major component of the diet for the billions of people as well as contributes to the animal feed and industry.

Greater yam belongs to genus *Dioscorea* of the family Dioscoreaceae under monocotyledons and are commonly known as yams. Out of the six commercially important edible species of yams, greater yam (*Dioscorea alata* L.) popularly known as *Ratalu*, is the most important edible yam in many parts of the world. Greater yam has a chromosome number of $2n=4x=40$, a natural tetraploid and is a native of South-eastern Asia. Greater yam has superior characteristics like high yield potential (especially under low and average soil fertility), ease of propagation and higher shelf life of tubers. It is a sun loving plant and commercially propagated vegetatively through tubers. Many of the *Dioscorea* species serve as a ‘life saving’ plant group for the marginal farmers and forest dwelling communities, during periods of food scarcity. In India it is extensively cultivated in Madhya Pradesh, North-eastern states, West Bengal, Bihar, Odisha, Uttar Pradesh, Kerala, Tamil Nadu, Gujarat and Maharashtra as a commercial crop. In Gujarat, it is mainly cultivated in the districts of South and middle Gujarat. The edible portion is mainly rich in carbohydrates along with a good amount of minerals. It contains 18-20% starch with a mucilaginous substance and is extracted on a commercial basis. It also contains quite a good amount of alkaloids and steroids having pharmaceutical value and is used in Ayurvedic, Unani and Homoeopathy

medicinal preparations. Besides, it is also ideal for fries, chips and flakes. Crop bio-fortification is a process which involves concentration of target nutrients in plants. By clonal propagation, we can choose parent line with a naturally high concentration of the target nutrient, which increases the availability of the nutrient to fight against malnutrition (Muthulisi et al., 2020). Besides the challenges, biofortified plants holds a bright future to address the malnutrition challenge (Garg et al., 2018).

The All India Coordinated Research Project on Tuber Crops (AICRP TC) is running in the Department of Vegetable Science, ASPEE College of Horticulture, Navsari Agricultural University (NAU), Navsari, Gujarat, has conducted a multi-location trial (MLT) on Gujarat Greater Yam-1 and it was evaluated during two seasons, viz., 2016-2017 and 2018-2019 at Navsari and Wagha centre. The experiment was conducted in a randomized block design (RBD) with three replications. The spacing adopted was 90×90 cm. The purple flesh variety of greater yam was clonally selected and biochemically analyzed at Central Instrument Laboratory, Soil Science & Agricultural Chemistry, N.M.C.A., NAU, Navsari. The ISSR profile of 27 greater yam genotypes including the genotype, NGy-7 (Gujarat Greater Yam-1: Hemlata) was generated by using the primer, UBC-808 and the RAPD profile using the primer OPB-6. The twenty seven genotypes used for the study are given below:

1. NGy-1	2. NGy-2	3. NGy-3	4. NGy-4
5. NGy-5	6. NGy-6	7. NGy-7	8. NGy-8
9. NGy-9	10. NGy-10	11. NGy-11	12. NGy-12
13. NGy-13	14. NGy-14	15. NGy-15	16. NGy-16
17. NGy-17	18. IGDa-2	19. IGDa-3	20. IGDa-4
21. Da-11	22. Da-25	23. Sree Roopa	24. Da-199 (Sree Karthika)
25. TRC	26. Sree Keerthi	27. Konkan Ghorkand	

Hemlata’s plant type is climbing, skin colour of tuber is dark brown, shape of tuber is long and flesh colour of tuber is dark purple. It had recorded an average tuber yield of 18.48 t ha⁻¹ which was 28.24% higher than the national check (NC) variety, Sree Karthika (14.41 t ha⁻¹). The purple flesh tuber of this clone is rich in total soluble sugars, crude fibre, anthocyanins, as well as the minerals phosphorus, potassium, ferrous, zinc and copper and

low in the anti-nutritional factor Diosgenin as compared to Sree Karthika (Table 1). The composition of the tuber on fresh and dry weight basis was as follows: anthocyanin (0.76 and 2.07 mg g⁻¹, respectively), crude fibre (1.14 and 3.20%, respectively), phosphorus (3.17 and 9.02%, respectively), potassium (5.84 and 17.53%, respectively), ferrous (0.80 and 2.53 mg kg⁻¹, respectively), zinc (0.08 and 0.23 mg kg⁻¹, respectively) and copper (0.07 and 0.20 mg kg⁻¹, respectively). The total soluble sugars (1.68%) was higher on fresh weight basis. It also recorded a total soluble sugar of 4.29% on dry weight basis.

Anthocyanins are water soluble pigments which play a significant role in reproduction by attracting pollinators and act as protectants against biotic and abiotic stresses. In Gujarat Greater Yam-1 (Hemlata) variety, anthocyanin content was higher *i.e.*, 0.76 mg g⁻¹ on fresh weight basis and 2.07 mg g⁻¹ on dry weight basis than the variety Sree Karthika which had 0.31 and 1.01 mg g⁻¹ respectively, of total anthocyanins.

These days, horticulture is moving from producing more quantity of food crops to producing nutrient-rich edible crops in sufficient quantities which will help in fighting the hidden hunger or micronutrient malnutrition especially in developing countries, where diets are dominated by micronutrient-poor staple food crops. Nutritional targets for biofortification include elevated mineral content, improved vitamin content, increased essential amino acid levels, better fatty acid composition, and heightened antioxidant levels in crops which are consumed by the poverty-stricken population of the world, which can significantly improve the amount of nutrients consumed by this target population (Garg et al., 2018). Food security, as defined by the Food and Agriculture Organization (FAO) of the United Nations,

exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life (Cathie et al., 2011). From Table 1, it is clearly seen that Gujarat Greater Yam-1 variety released as in the name 'Hemlata' contains appreciable amounts of minerals in the tubers (Fig. 1).



Fig. 1. Anthocyanin rich tuber of Gujarat Greater Yam-1 (Hemlata)

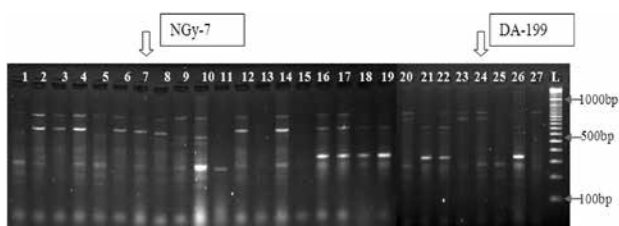
Tab. 1. Proximate composition and quality parameters (on fresh and dry wt. basis) of Gujarat Greater Yam-1 and Sree Karthika

Sl. No.	Parameter	Gujarat Greater Yam-1		Sree Karthika (NC)	
		fresh wt. basis	dry wt. basis	fresh wt. basis	dry wt. basis
1.	Total carbohydrates (%)	21.17	60.65	23.83	85.34
2.	Starch (%)	19.13	55.00	21.02	79.67
3.	β-carotene (μg g ⁻¹)	0.65	1.88	0.67	1.78
4.	Total soluble sugars (%)	1.68	4.29	1.24	5.11
5.	Crude fibre (%)	1.14	3.20	1.13	3.05
6.	Total anthocyanins (mg g ⁻¹)	0.76	2.07	0.31	1.01
7.	Phosphorus (%)	3.17	9.02	2.81	8.54
8.	Potassium (%)	5.84	17.53	4.18	13.83
9.	Fe (mg kg ⁻¹)	0.80	2.53	0.28	0.83
10.	Zn (mg kg ⁻¹)	0.08	0.23	0.07	0.18
11.	Cu (mg kg ⁻¹)	0.07	0.20	0.03	0.10
12.	Diosgenin (%)	0.09	0.28	0.14	0.93



RAPD profile of 27 greater yam genotypes generated by using primer OPB-6

Fig. 2. DNA finger printing of genotype NGy-7 (Gujarat Greater Yam-1: Hemlata) with primer OPB-6



ISSR profile of 27 greater yam genotypes generated by using primer UBC-808

Fig. 3. DNA finger printing of genotype NGy-7 (Gujarat Greater Yam-1: Hemlata) with primer UBC-808

The results are in agreement with the findings of Cathie et al., (2011), Gomathi et al., (2017), Garg et al., (2018), Parulekar et al., (2019) and Muthulisi et al., (2020).

In DNA Fingerprinting analysis, many cultivars showed unique fingerprint patterns indicating the utility of DNA finger printing in cultivar identification. DNA fingerprinting profile of greater yam genotypes was carried out which is depicted in Fig. 2 and Fig. 3. It showed that genotype NGy-7 (Hemlata) is genetically distinct from the check variety, Sree Karthika (DA-199).

Combating micronutrient malnutrition is considered to be amongst the best investments that generate a high return socio-economic benefits according to 2008

Copenhagen consensus. The consensus listed bio-fortification, a method of breeding crops to increase their nutritive value, as one of its top five investments to address global challenges. Diversified and highly nutritive tuber crops are an affordable source for the poor people in the maintenance of their health and prevention of disease and have great potential in handling malnutrition and hunger issues. The nutrient rich bio-fortified greater yam variety, 'Hemlata' which was recommended for commercial cultivation for the state of Gujarat is possibly one of the ecofriendly and cost effective solution of malnutrition. Development as well as production and consumption of bio-fortified tuber crops needs to be popularized for preventing and controlling various health related issues also. So, this variety can help to achieve this goal to some extent.

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Ayemor, G. S. 1985. Effect of retting of cassava on product yield and cyanide detoxification. J. Food Technol., 20: 89-96.

Bates, L. S., Waldren, K. P. and Teare, I. D. 1973. Rapid determination of free proline for water stress studies. Pl.Soil, 36:205.

Jackson, M. L. 1973. Soil Chemical Analysis. New Delhi: Prentice Hall of India Pvt. Ltd.

Henshaw, G. G. 1982. Tissue culture methods and germplasm storage. In: Plant Tissue Culture. Fujiwara, A. (Ed.). Maruzen, Tokyo. pp. 789-792.

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