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Calcium nutrition influencing mineral content, nitrogen assimilation, flowering and yield of dragon fruit (*Selenicereus monacanthus*)

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Abstract
Dragon fruit is considered as one of the important tropical fruit crops due to its nutritional significance. Under acidic soil condition, sustaining crop production is one of the major challenges. The present investigation aimed at assessing the efficacy of calcium nutrition on mineral composition, nitrogen assimilation, flowering and yield of dragon fruit under acidic soils of eastern tropical region. The treatments comprised of soil application of calcium @ 100, 200, 300, 400 and 600g hill^{-1} year^{-1}. Calcium-absentia was considered as a control. Application of Ca @ 400g hill^{-1} year^{-1} exhibited the highest acquisition of minerals such as N, K, Ca, Zn, B and Cu in the matured old and recently matured shoots. Whereas P, Mg, Fe and Mn were largely attained with Ca @ 200 g hill^{-1} year^{-1}. Maximal chlorophyll content, total carbohydrate, nitrate reductase activity, nitrogen use efficiency, highest fruit set percentage and overall fruit yield were also recorded with Ca @ 400 g hill^{-1} year^{-1}. Significant correlation between the fruit yield and shoot calcium content, indicated the possible interplay of calcium in improving yield in dragon fruit through improved nitrogen assimilation and carbohydrate synthesis. The dose Ca @ 400 g hill^{-1} year^{-1} was conclusively considered as the optimal level of calcium nutrition for dragon fruit under acidic soils of eastern tropical regions. Furthermore, the level 600 g hill^{-1} year^{-1} did not result in significant appreciation of assessed variables and hence was adjudged as a supra-optimal dose.

Keywords: Calcium, dragon fruit *Selenicereus monacanthus*, mineral composition, total carbohydrate, nitrate reductase activity and yield

1. Introduction
Dragon fruit is a commercial fruit crop in India. It belongs to family cactaceae and genus *Selenicereus*. The crop is reported to have originated from Southern Mexico and presently, it has expanded globally in Central and tropical South America, Southeast Asia and China (Wichienchot *et al.*, 2010) [33]. The potential popularity of the crop has provided it a specific niche in international markets. The fruit crop has significance with respect to its nutraceutical properties and pigment betalains which has a tremendous scope in food industries (Lourith and Kanlayavattanakul, 2013) [21]. The-red fleshed dragon fruit (*Selenicereus monacanthus*) is preferably gaining momentum in Indian markets due to its attractive colour and health benefits. The commercial significance of the fruit crop, necessitates plentiful production of quality fruits for which mineral nutrition is one of the most widely adopted package of practices. Plants require various mineral ions as essential nutrients (Grusak *et al.*, 2016) [11]. Each mineral nutrients play diverse and fundamental roles in maintaining the plant growth and development. Under acidic soil condition, fruit production is often constrained due to unavailability of mineral ions. Calcium as an essential element play a vital role in regulating the transport of other nutrients into the plant system under acidic soil conditions (Tian *et al.*, 2016) [31].

Calcium is a secondary cationic element which caters a variety of functions within the plant system. It primarily maintains cell structure, integrity and cellular polarity. It plays an important role in plant growth and development and is involved in numerous cellular metabolism and transcriptional regulation (Khushboo *et al.* 2018) [20]. The element has been recognized as a secondary messenger, a cytosolic signaling molecule. It binds and activates calmodulin, which is a calcium-binding, poly functional messenger protein mediating plant response to phytohormones, biotic and abiotic stresses (Silva *et al.* 2011) [28]. The element also has an ameliorative effect and benefit crops under conditions of salinity and mineral toxicity (Reyes-Diaz *et al*., 2011) [27]. Besides, aiding in plant growth and development, role of calcium in enhancing yield has also been commonly reported (Hosseini *et al*., 2021) [15].

The mineral calcium can be applied to soil in form of calcium chloride, calcium nitrate, calcium sulphate and lime (Jenkins and Mahmood, 2003) [18]. However, calcium nitrate has been reported to be highly soluble and more effective in increasing Ca concentration in soil solution (Ahmad *et al*., 2009) [1]. Foliar application of calcium may not be as efficacious as soil application for enhancing crop yield, as the bulk of calcium is up taken primarily through xylem and calcium exhibits poor mobility in phloem (White and Broadley, 2003) [34]. Additionally, in dragon fruit, calcium nutrition is pertinent as the mineral constitute an integral component of fruit nutritional composition (Nerd *et al*., 1999) [23]. Implication of soil application of calcium in influencing the mineral dynamics, flowering and yield of dragon fruit has not been studied. Hence, the present investigation is aimed at assessing the influence of calcium nutrition through soil application on mineral acquisition, nitrogen assimilation, flowering and fruit yield of red-fleshed dragon fruit (*Selenicereus monacanthus*) under acidic soil condition of eastern tropical regions of India. It is hypothesized that soil application of calcium can be one of the effective yields enhancing management strategy in dragon fruit under acidic soil conditions.

2. Material and Methods

The experiment was conducted at the Central Horticultural Experiment Station (ICAR-IIHR), Bhubaneswar, India, located at latitude: 20°27′ N; longitude: 85°40′ E, during two growing seasons in the year 2020 and 2021. The region represents a tropical wet and dry climate with an annual average temperature of 26.7 °C, relative humidity of 77.8% and annual rainfall of about 1500 mm. The soil of the locality is sandy loam, strongly acidic in reaction with low organic carbon content. The soil is deficit in nitrogen, calcium, magnesium, sulphur and boron content. While, potassium content is moderate. Conversely, the levels of phosphorous, iron, zinc, copper and manganese in soil are high. The experiment was carried out on 4-years old dragon fruit (*Selenicereus monacanthus*), a red-fleshed strain (CHES-B-DF-1). The plants were trained on single post system with each post (hill) comprising of 4 plants. The crops in the experiment field were provided with uniform management practices pertaining to nutrient and disease management.

2.1 Treatments and experimental design

Healthy dragon fruit plants of uniform growth and vigour were selected for imposing the treatments. Calcium fertilization was done in form of Calcium nitrate (N- 15.5% and Ca- 19%) applied in the rhizosphere region followed by irrigation. The treatments comprised of increasing levels of calcium, with Ca-0 as control (Calcium absence) and Ca-100, Ca-200, Ca-300, Ca-400, Ca-600 indicating the application of calcium @ 100, 200, 300, 400 and 600g hill^{-1} year^{-1}, respectively. The treatments were imposed in a randomized block design, each comprising of four replications, further each replication consisted of 12 plants (3 hills). Considering, the soil fertility status of the experimental site, a basal dose of primary fertilizer consisting of nitrogen (500g hill^{-1} year $^{-1}$), given in the form of urea (N - 46%), phosphorous (200g hill^{-1} year $^{-1}$) in the form of diammonium phosphate (P_2O_5 - 46%; N - 18%) and potassium (300g hill^{-1} year $^{-1}$) in the form of muriate of potash (K_2O – 60%) was also applied. All of these fertilizers including calcium nitrate were applied in the month of March, June and September in three equal splits

2.2 Flowering, fruit set and yield

Dragon fruit bear fruits in flushes, hence the data on number of floral buds and yield per pole was quantified as the total sum of all the flushes, which occurred over the entire fruiting season. The floral buds were counted seven days after emergence, when it attained a sizeable shape. Fruit set occurs upon successful pollination and usually occurs in 18-20 days of floral bud emergence. The fruit set percentage was estimated as the (number successful flowers which advances further development into fruit/total number of floral buds multiplied by 100 and expressed as per centage. The yield was estimated for each flush and the average was worked out and expressed in kg hill^{-1}.

2.3 Mineral content and bio-chemical estimation

The mineral content of matured and recently matured shoots was estimated. The samples were oven dried at 65 ° C for 48 hours followed by grounding and sieving before analysis. The samples were subjected to wet digestion by using a mixture of HNO_3 and $HClO_4$ in the proportion (4:1) according to the protocol laid down by Zasoski and Burau (1977) [36]. The mineral content *viz.*, P, K, Ca, Mg, Fe, Mn, Zn, B and Cu were estimated by inductively coupled plasma optical emission spectrometry ICP-OES (Perkin Elmer, Avia 200). The nitrogen content (%) was determined by an Organic Elemental Analyzer Unicube (Elementer, mode-CHNS). The chlorophyll content of recently matured shoots, which are the putative productive shoots was estimated by the protocol laid down by Fan *et al*., 2013 [8]. For estimation of chlorophyll content, the samples were extracted with 80% acetone and absorbance (OD) was measured with a spectrophotometer (Eppendorf, Biospectrophotometer® Fluorescence) at 663 nm (OD_{663}) and 645 nm (OD_{645}) wavelength and the total chlorophyll (mg g^{-1}) was quantified by using following formulae:

$$Total\ Chll = 20.2(A645) + 8.02(A663)\ x\ \frac{V}{1000\ x\ W}$$

Where, V= volume made up, W= weight of the sample taken

Total carbohydrate was estimated by phenol-sulphuric acid method following the protocol of Nielsen, 2017 [22]. The absorbance of the reaction mixture was measured at 490 nm. The total carbohydrate content in the sample was expressed in µg ml^{-1}. The nitrogen assimilation was quantified by estimating the activity of enzyme Nitrate reductase. The *in vivo* assay of NR activity in leaf was done according to the procedure of Hageman and Hucklesby (1971) [13] with slight modifications. The incubation medium comprised of 0.05M potassium phosphate buffer (pH-7.8) and 0.4M KNO3 solution. The contents were incubated in water bath at 35ºC for 75 min under dark conditions and nitrite content was estimated by the method of Evans and Nason (1953) [6]. The enzyme activity was expressed as µmole NO_2 g^{-1}(fw)hr $^{-1}$. The contribution of nitrogen application to dragon fruit yield was calculated as Nitrogen Use Efficiency (NUE) as described by Du *et al*., (2017) [4].

NUE= Y/N, Where, NUE is measured in Kg Kg^{-1}, and N is the amount of nitrogen applied (Kg $hill^{-1}$) and Y= Entire fruit yield (Kg) $hill^{-1}$.

2.4 Statistical Analysis

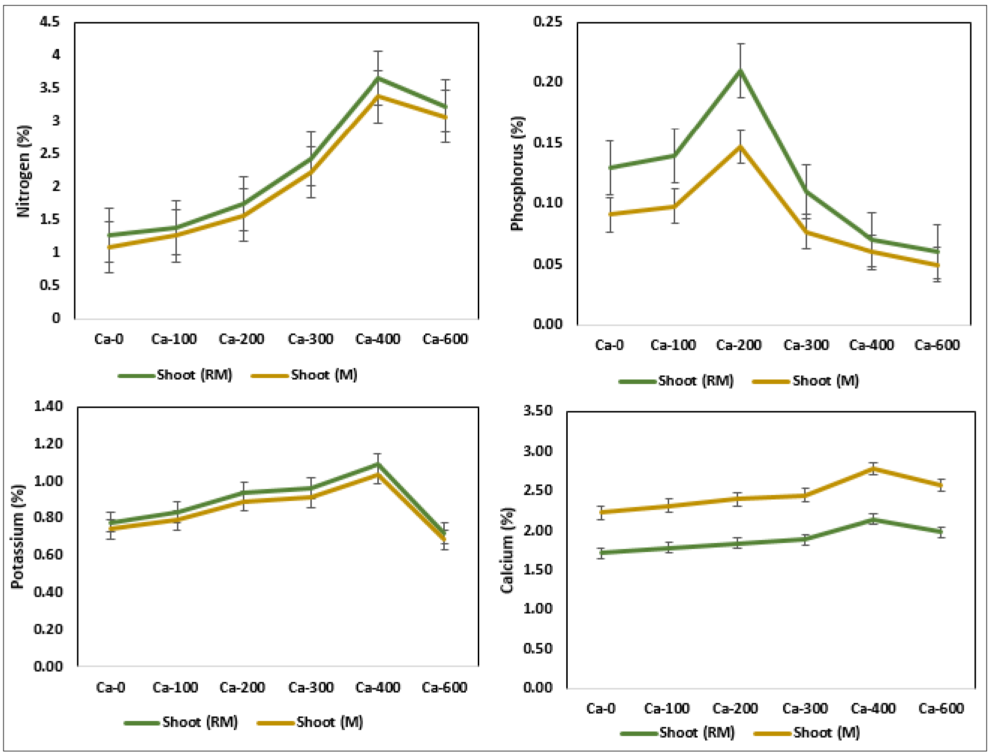
Analysis of variance (ANOVA) was performed to test the significant differences among means of various attributes using OPSTAT statistical software and the means were compared using Tukeys' HSD test ($p \leq 0.05$). The data for both the years was averaged to determine comparative responses of calcium nutrition on the assessed parameters.

3. Results and Discussion

3.1 Influence of Calcium fertilization on shoot mineral content

The data presented in Figure 1, illustrates a significant effect of calcium fertilization on mineral content of dragon fruit shoots. It is evident that the acquisition of minerals such as N, K, Ca, Zn, B and Cu exhibited an increasing trend with increasing calcium rates. The treatment calcium @ 400g $hill^{-1}$ $year^{-1}$ exhibited the maximal content of these minerals, however, increasing the calcium level to 600g $hill^{-1}$ $year^{-1}$ manifested a decline in majority mineral contents. The attainment of mineral such as P, Mg, Fe and Mn were maximum at comparatively lower calcium level (200g $hill^{-1}$ $year^{-1}$). Similar trend was exhibited in both matured old and recently matured shoots. It was also noticeable that the attainment of mineral such as N, P, K is more in recently matured shoots and the acquisition of secondary and micronutrient is more in old matured shoots. Similar findings have been reported by Ramirez-Builes *et al.* (2020) [26]. Calcium regulates ion transport and controls ion-exchange behaviour in the plant system (Hadi and Karimi, 2012) [12]. Enhanced K^+ and Ca^{2+} concentration with application of calcium nitrate has been reported by Ebert *et al.*, (2002) [5]. Studies by Ahmad *et al.*, (2009) [1] presented that calcium nitrate is highly soluble and effective in increasing calcium ion concentration in soil solution. It is likely the altered calcium ion status in soil solution might have impacted uptake of other mineral ions. Similar findings have been reported by Xing *et al.* (2021) [35], indicating increased Ca accumulation rate in the plant tissue with increasing Ca supply levels. Higher calcium content in the matured shoot can be attributed to accelerated uptake of calcium ions in the xylem due to the transpiration pull. Further a reduced calcium content was observed in recently matured leaves than matured old leaves as calcium deficiency symptoms are comparatively more observed in younger tissues (Tang and Luan, 2017) [30].

The contribution of calcium in nitrogen uptake specifies that both deficit and excessive calcium levels in soil can inhibit N absorption (Xing *et al.*, 2021) [35]. The shoot N content of the control treatment was lower than the other fertilization treatments because of the variable N application rate as a result of varying calcium nitrate administration. Poljak *et al.*, (2007) [24] showed that Ca fertilizers increase P concentrations in the plant. However, in present study, increasing calcium rates imposed an antagonistic effect on P-content in shoot, likely due to precipitation of phosphate ions by excessive calcium ions (Jakobsen, 1993) [17]. The calcium-based protein sensors regulate the uptake and translocation of several mineral ions such as potassium and magnesium (Tian *et al.*, 2016) [31]. Higher magnesium content in shoots at lower calcium levels in soil illustrates the significance of magnesium ions accumulation in mesophyll as a substitute of Ca^{+2} to combat the osmotic role (Gilliham *et al.*, 2011) [10]. Similar findings have been reported by Khayyat *et al.*, (2009) [19], who reported high levels of copper and zinc concentrations in shoots as induced by calcium application. In the present study, pattern of iron and manganese uptake followed a similar trend, indicating a positive interrelation as both Fe and Mn are mobilized by similar root processes (Warden and Reisenauer, 1991) [32]. The presence of calcium ions in soil also plays an important role in controlling the severity of specific ion toxicities (Hue *et al.*, 2001; Fageria and Baligar, 2008) [16, 7].



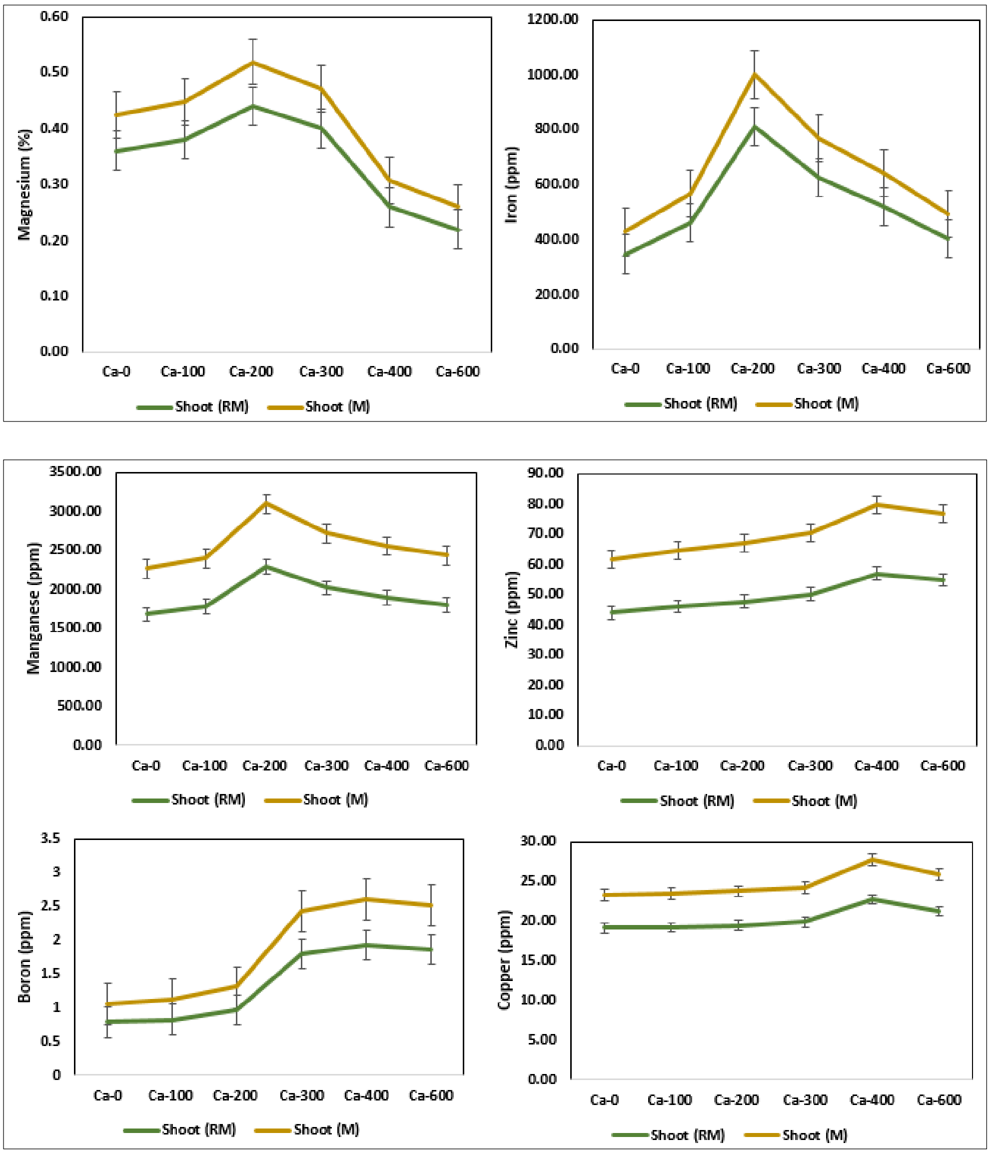


Fig 1: Influence of calcium nutrition on mineral content of dragon fruit shoot (RM-Recently matured shoot, M-matured old shoot)

3.2 Influence of calcium fertilization on shoot chlorophyll, carbohydrate content, nitrate reductase activity and nitrogen use efficiency

The perusal of data in table 1, indicates that the calcium treatment had significant influence on the total chlorophyll content of recently matured shoots. The treatment Ca @ 400g hill-1year-1 manifested the maximal content of chlorophyll (~2-fold increase over control). Additionally, with increasing the calcium level beyond 400g hill-1year-1, a subtle decline in chlorophyll content was observed. Significant correlation existed between total chlorophyll content and calcium content in recently matured shoot (*r*= 0.872**). Similar findings have been reported by Qeyami *et al*., 2020 [25]. Additionally, total chlorophyll content in shoot also exhibited good correlation with leaf nitrogen content (*r*= 0.803**). Bojović *et al*., 2009 [3], also indicated the possibility of close linkage between chlorophyll and nitrogen content.

The total carbohydrate content in the shoot was also maximally attained with the application of calcium @ 400g hill^{-1}year^{-1} (~37% increase over control). However, a further increase in calcium level (600g hill^{-1}year^{-1}) demonstrated a decline in shoot carbohydrate content. There existed a significant correlation between shoot carbohydrate content with chlorophyll content (*r*= 0.899**) and nitrogen use efficiency (*r*= 0.985**). Positive implication of calcium application on carbohydrate content has been reported by Singh *et al*., (2018) [29]. The activity of enzyme nitrate reductase increased substantially with elevated calcium levels. The highest activity was recorded in the treatment Ca @ 400g hill^{-1}year^{-1} (~1.5 times higher than control). Further increasing the calcium fertilization level to 600g hill^{-1}year^{-1} manifested a decline in nitrate reductase activity. The enzyme nitrate reductase helps in nitrogen assimilation in plant tissues as it catalyzes the first step in reduction of nitrate form of nitrogen to organic forms and its activity reflects the level of N content in plant (Gerik *et al*., 1998) [9]. Additionally, the shoot nitrogen content exhibited significant correlation with nitrate reductase activity (*r*= 0.89). Elevated activity of nitrate reductase enzyme with calcium treatment has also been reported by Singh *et al*., (2018) [29].

Likewise, the Nitrogen Use Efficiency (NUE), manifested an increasing trend with increasing levels of calcium application in the soil, with highest efficiency being recorded in the treatment Ca @ 400g hill^{-1}year^{-1}. It is likely that calcium application improves nitrogen uptake resulting in improved NUE (Akram *et al*., 2013) [2]. Consequently, the treatment Ca @ 400g hill^{-1}year^{-1} recorded highest no. of flower buds, enhanced fruit set and maximal yield (~ 2-folds, 39% and 2-times increase over treatment control, respectively). The fruit yield in dragon fruit established significant correlation with total carbohydrate content in shoots (r= 0.98) and NUE (r= 0.99). There also existed significant correlation between yield and shoot calcium content, indicating the possible role of calcium in improving fruit yield in dragon fruit through improved nitrogen assimilation and carbohydrate synthesis. Similar findings have also been reported by Helal *et al*., (2015) [14].

Table 1: (Influence of calcium fertilization on shoot chlorophyll content, total carbohydrate, nitrate reductase activity, nitrogen use efficiency, flowering and fruit yield of dragon fruit)

Treatments	Total chlorophyll (mg g^{-1})	Total carbohydrate (µg ml^{-1})	Nitrate reductase activity (µmole NO_2 g^{-1} fwhr^{-1})	NUE (Kg kg^{-1})	No. of flower buds	Fruit set (%)	Yield (Kg hill^{-1})
Ca-0	0.26^{c}	175.34^{c}	2.65^{c}	25.10^{e}	22.32^{d}	44.67^{c}	2.91^{e}
Ca-100	0.39^{bc}	186.52^{c}	2.77^{c}	33.21^{d}	28.45^{cd}	50.78^{bc}	3.67^{de}
Ca-200	0.42^{ab}	201.36^{bc}	2.89^{bc}	45.31^{c}	33.56^{bc}	52.1^{bc}	4.15^{cd}
Ca-300	0.48^{ab}	225.75^{ab}	3.02^{bc}	52.12^{bc}	38.35^{ab}	57.87^{ab}	4.89^{bc}
Ca-400	0.55^{a}	240.91^{a}	4.21^{a}	65.15^{a}	46.51^{a}	62.12^{a}	5.85^{a}
Ca-600	0.43^{ab}	229.83^{a}	3.24^{b}	55.09^{b}	32.34^{bc}	53.45^{b}	5.12^{ab}
CD	0.123	24.100	0.342	6.040	7.676	6.907	0.757
SEM	0.030	5.874	0.083	1.472	1.871	1.683	0.184

*Mean values followed by same letter in each column show non-significant difference

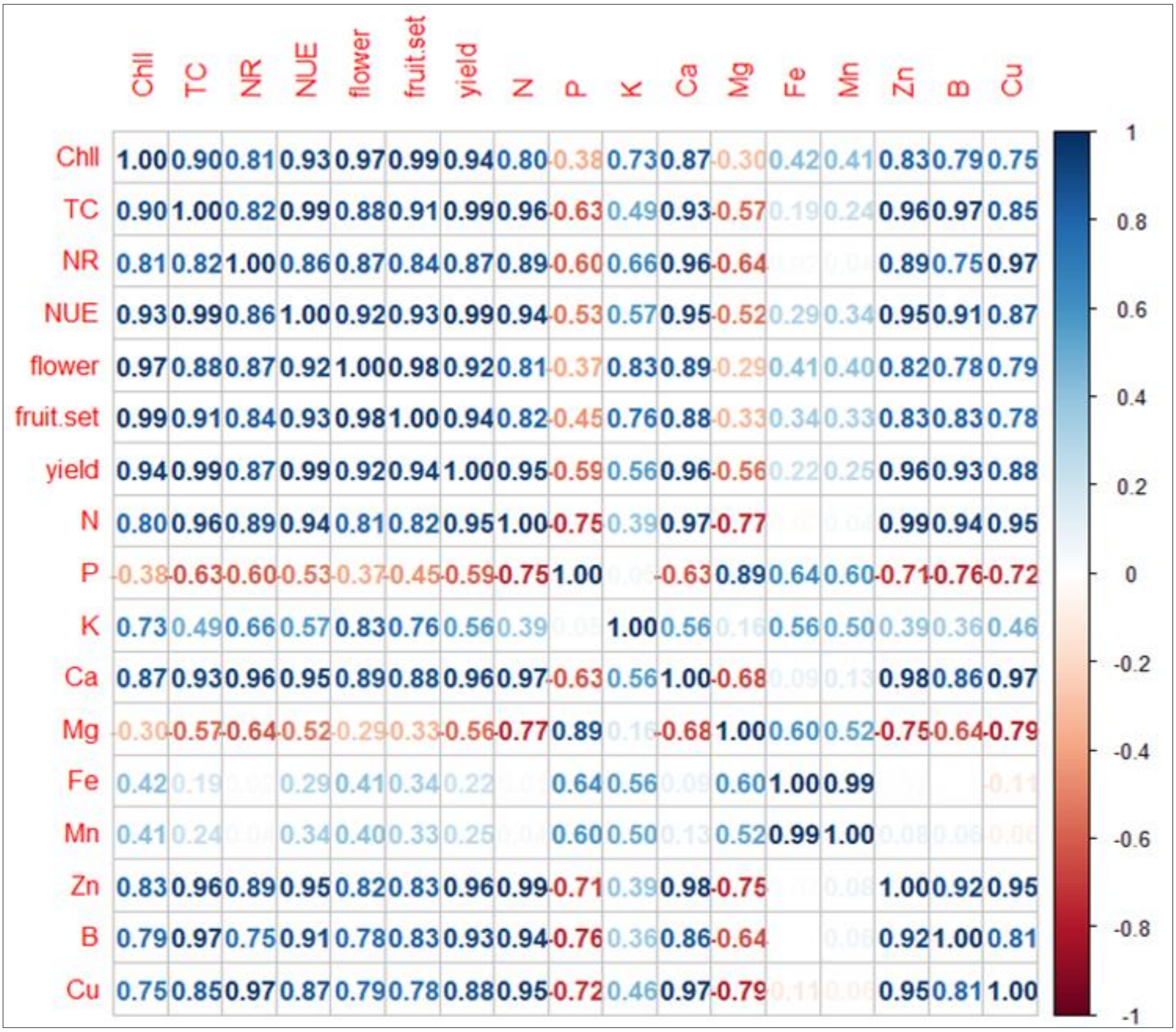


Fig 2: Correlation coefficient values among the variables (Chll- Total chlorophyll, TC- Total carbohydrate, NR- Nitrate Reductase activity, NUE- Nitrogen Use Efficiency) Positive correlations are displayed in blue and negative correlations in red colour

4. Conclusion
Calcium application in dragon fruit grown under acidic soil condition proves beneficial in terms of mineral nutrient acquisition. The calcium nutrition also enhanced shoot chlorophyll, carbohydrate content, nitrate reductase activity and nitrogen use efficiency. Consequently, the flowering, fruit setting and overall yield also witnessed an enhancement with increasing rates of calcium administration until the dose Ca @ 400g hill^{-1}year^{-1}. It was also evident that application of calcium at optimal level was desirable as the higher dose Ca @ 600g hill^{-1}year^{-1} did not result in enhancing yield and yield attributing characteristics in dragon fruit under acidic soil conditions of eastern tropical region.

5. Acknowledgements
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6. Conflict of interests
The authors have declared no conflict of interests exist.

7. Data Availability Statement
Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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