MANAGEMENT OF CALCIUM, MAGNESIUM, SULPHUR AND BORON IN TC BANANA (*Musa* spp.) var. NENDRAN

by

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DECLARATION

I, hereby declare that this thesis entitled **"MANAGEMENT OF CALCIUM, MAGNESIUM, SULPHUR AND BORON IN TC BANANA (*Musa spp*.) var. NENDRAN"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"MANAGEMENT OF CALCIUM, MAGNESIUM, SULPHUR AND BORON IN TC BANANA (*Musa* spp.) var. NENDRAN"** is a record of research work done independently by Ms. Neethu Prabhakar (2013-11-116) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Introduction

1. INTRODUCTION

Banana (*Musa* sp.) is the cheapest, plentiful and most nourishing fruit crop of the world. It is grown in more than 130 countries with a total annual production of 97 million tones of fruits. It one of the oldest fruit crops cultivated by man and it is the world's most important fresh fruit commodity in terms of volume of trade. Bananas and plantains are the only group of fruits that constitute a staple food for millions. They are consumed fresh, cooked, steamed, roasted and brewed.

India is world's leading producer of banana with an annual production of 297.24 lakh tones which is contributed by 11.1 percent of total fruit area of the country and accounts for 33.4 percent of total fruit produced. The productivity of the crop is 37.0 t/ha. In Kerala banana is grown in 34.46(000'ha) with a production of 528.21(000'MT) and productivity of 15.32 t/ha (Saxena, 2015).

In vitro multiplication of banana is being widely used for the commercial scale production of large number of homogenous, high yielding, pest and disease free plantlets. Micropropagation of banana is now done in industrial level and annually upto 50 million tissue cultured plants are produced worldwide (Tiesson and Cote, 1997). Nendran (AAB) is a popular variety of banana extensively cultivated in Kerala which is relished as fruit as well as used for processing. Tissue culture is an important tool in meeting the growing demand of quality planting material in our country. Banana being a high nutrient demanding crop requires adequate quantities of nutrients for maximizing the yield and improving the quality characteristics.

According to Borges (2004), K and N are the nutrients most taken up and required for growth and yield of the banana plant, followed by Mg and Ca. Calcium has an important role in maintaining the structure and permeability of cell membrane, it is essential for cell elongation and division. Even a temporary

shortage of calcium can cause the development of deformed leaves and leads to production of low quality fruits. Magnesium is a major constituent of chlorophyll, it also serves as a structural component of ribosomes. Deficiency of magnesium causes interveinal chlorosis, changes in phyllotaxy, purple mottling of petioles, and separation of leaf sheaths from pseudostem. Sulphur is important in stabilizing and determining the configuration of proteins. It is needed for the synthesis of metabolites like coenzyme A, biotin, thiamine etc. As a result of sulphur deficiency, leaves become whitish yellow, stunted growth and formation of small or choked bunches. Boron is needed for the development of new cells, translocation of starch and sugars, regulation of carbohydrate metabolism. Boron deficiency symptoms include leaf curling and deformation, presence of white stripes perpendicular to the veins on the underside of the lamina.

Kerala soils are characterized as the end products of intense weathering processes under the humid tropical environment which resulted in the formation of strongly acidic sesquioxides rich soils with poor base content and low native fertility.

Soils with low activity clays are inherently acidic and contain very small amounts of exchangeable Ca and Mg. Aluminium toxicity coupled with Ca and Mg deficiency occur in about 70% of the acid, infertile regions of the tropics (Varghese and Byju, 1993). Jose *et al*. (1993) have identified the physical and chemical constraints of laterite soils of Kerala for crop production. Soil erosion, hardening of laterite at the surface, low water holding capacity, reduced effective soil volume due to concretions, and drought stress have been cited as the major physical constraints. High soil acidity, high exchangeable Al, low CEC, high AEC, low levels of Ca and Mg along with P deficiency are the main chemical constraints.

Arihara and Srinivasan (2001) reported that deficiency of secondary nutrients including sulfur is an important factor reducing nitrogen and

phosphorus use efficiency and is becoming more widespread in cropping systems worldwide.

Indian soils exhibit wide degree of S deficiency because of increased crop removal accentuated by increased use of S free fertilizers, liming, phosphatic fertilizers and highly imbalanced use of fertilizer nutrients. Due to lack of replenishment and leaching losses the coarse textured soils, highly weathered soils like Ultisols, Oxisols, Alfisols and Inceptisols are deficient in S. Some soils rich in organic matter may still have lower available S because of higher adsorption and immobilization. Such soils respond to applied S (Kanwar, and Mudahar, 1986).

Sulphur deficiency is widespread and is becoming an important constraint for sustainable development in agriculture. Now sulphur is emerging as the fourth most important nutrient in terms of extensiveness of deficiencies in India.

Boron is an important micronutrient for crops because of its role in the cell wall synthesis, root elongation, sugar translocation, nucleic acid metabolism, lignifications and tissue differentiation. Coarse-textured acid soils of humid and perhumid regions, calcareous soils and those with low organic matter content are more prone to B deficiency. In India, deficiency of B is more widespread next to zinc.

In modern day agriculture, challenge is to sustain the soil fertility in cropping systems operating at high productivity levels. To achieve this objective, innovative management alternatives will be essential for nutrient management. Due to the long term continuous application of straight fertilizers alone to the field the importance of application of secondary nutrients and micronutrients often gets neglected and the deficiencies of these nutrients are being increasingly reported from various parts of the state.

Hence the main objective of the study is to investigate the following objectives

1. To study the effect of hardening of tissue culture banana using fortified potting mixture containing calcium, magnesium, sulphur along with boron foliar sprays

2. To study the effect of application of calcium, magnesium, sulphur and boron on tissue culture banana cv. Nendran on various yield and quality parameters.

Review of literature

2. REVIEW OF LITERATURE

2.1. BANANA

Banana is the fourth most important food crop in the world after rice, wheat and maize. The fruit is both a staple food and an export commodity. Banana is a general term that refers to all wild species, landraces and cultivars belonging to the family *Musaceae*, genus *Musa* (Ortiz, 2008). The genus *Musa* has more than 50 species, with some of these species having numerous subspecies.

Bananas (*Musa* spp.) are cultivated in over 130 countries, and are one of nature's best known sources of K and one of the most convenient and nutritionally dense food items. They are also good and inexpensive sources of vitamins A, C, B_6, and minerals. In addition to the well-known effects of K in lowering the risk of developing diseases such as heart attack and strokes, functional compounds in banana are reported to relieve constipation, heartburn, ulcers, and have been linked to prevention of anaemia by stimulating the production of haemoglobin in the blood (Robinson and Sauco, 2010).

Plantain (*Musa* sp. AAB) is one of the important staple foods in the tropical and sub-tropical regions of the world (Englberger *et al*., 2006). The fruit is an important source of carbohydrate, vitamins, proteins, potassium, iron, calcium, carotenes and ascorbic acid and also contains moderate amounts of thiamine, riboflavin, nicotinic and folic acid (Rasheed, 2003). Per capita annual consumption is as high as 150 kg in some traditional production areas of West and Central Africa (Vuylsteke *et al*., 1997).

Plantains require high amounts of nutrients for optimum growth and fruit production but these nutrients are often supplied in part by the soil (Lahav, 1995).

2.1.1. Tissue culture banana

In banana, the difficulty to obtain large number of uniform disease free plants with high yield potential by the conventional propagation of techniques is one of the major limiting factors in increasing productivity. Another important problem faced by the growers is the staggered flowering (variability in time of flowering). Tissue culture technology enables the rapid production of a large quantity of uniform disease free plants from a single plant showing good genetic potential (Sheela and Nair, 2001)

The physiological basis for the increased vigor and yield potential of TC plants is the juvenile nature of the material and the fact that there are no stored reserves, thus creating an increased photosynthetic demand. These conditions lead to increased photosynthetic efficiency, increased functional leaf area, increased root vigor and increased total dry mass. The plants cannot tolerate poor management, and furthermore, any early stress on either the leaves or roots is devastating to the plant because leaves and roots are interdependent on each other during the first three months of development (Robinson, 2000).

2.2. Nendran

The variety Nendran ranks first in commercial value. The cultivators of Agasthiamali ranges call this variety as “King of Banana”. The shelf life of the fruits of Nendran is more, compared to that of others. So, the fruits of Nendran have been exported to the Arabian and European countries (Das, 2010).

Venugobal (2008) stated that banana’s are mostly grown by small and marginal farmers either in homesteads or in well drained rice field and Nendran is the most popular commercial cultivar much loved by cultivators for its excellent fruit quality, sustained income and multiple uses ranging from being much valued for infants and culinary purposes to diverse processed products.

Fruit pulp of Nendran contains vitamins B_1, B_2, B_3, Vitamin C, amino acids, iron, calcium, phosphorus and proteins in substantial amount which are

needed for the daily diet of human beings (Das, 2010). Besides it is having several medicinal properties too.

2.3. Banana nutrition

Research on the mineral nutrition of bananas first involved the description of symptoms of plant deficiency and the determination of fertilizer rates in a range of soils (1920s-1970s) (Lahav, 1995). In the second stage (1960s-1990), it focused on the role played by nutrients in banana growth and development (Lahav, 1995), on soil-plant relationships with respect to cationic balance (Martin and Montagut, 1966).

Banana is a high nutrient demanding crop and for better growth and fruit production it requires high amounts of mineral nutrients which are often only partly supplied by the soil. *Twyford and Walmnsley* (1974) reported that to obtain a crop yield 50 t/ha/year of fresh fruit, about 1500 kg K_2O/ha/year may be extracted from the soil. Amounts of other nutrients found in field grown plants at harvest are (in kg/ha/year): N-450; P-60; Ca-215; Mg-140; B-1.25 respectively. Thus large quantities of nutrients have to be replaced in order to maintain soil fertility and to permit the continuous production of high yields

Bananas and plantains require large quantities of mineral nutrients to maintain high yields and these can only be supplied by growing on very fertile soils or by applying supplementary fertilizers. Only about 15% of bananas and plantains worldwide are intensively fertilized, the remainder being grown with organic matter, household refuse or nothing (Robinson, 2000).

Optimum leaf concentrations for plantains do not differ very much from those for bananas. Nutritional reference norms for Horn plantain leaves are as follows: N 2.7%; P 0.2%; K 4.3%; Ca 0.5%; Mg 0.3%; Zn 10 ppm; Cu 9 ppm; Mn 66 ppm; Fe 69 ppm; S 1 ppm (Rodriguez *et al*., 2007).

Low inherent soil fertility like soil organic matter, total N and the ratio K/(Ca + Mg) has been suggested to limit banana production (Wairegi *et al.,* 2010). P and Mg deficiencies were observed on highly weathered soils and K deficiencies dominate generally on soils that have a slower weathering rate or where it is inherently lacking due to the nature of the parent material (i.e. quartzite and granite) (Gaidashova *et al.,* 2009). Optimum K/Mg ratio as suggested by Delvaux (1995) is 0.3:1. Soil constraints (*i.e.* soil pH, K, Mg and Ca) were found to account for 67% of yield limitations to banana (cv. Cavendish) production in smallholder farms in central Kenya (Okumu *et al.,* 2011).

Magdoff (1995) reported the essentiality of the synchrony of plant-available soil nutrients and crop nutrient demand for optimum crop performance and environmental protection.

2.4. CALCIUM

2.4.1. Calcium in soils

Calcium in soils are derived primarily from the Ca bearing aluminosilicates like amphiboles and feldspars, Ca carbonates, and Ca phosphates. Adams (1974) reported that soil solution levels of Ca are usually the highest of all cations.

Depending upon the nature of parent material, degree of weathering and the prevailing climatic conditions, calcium content of different soil types varies significantly. Calcium content of the soils derived from limestone are usually higher and is in the range of 100-700 g Ca/kg soil. In calcisols exchange complex is completely saturated with bases; Ca^{2+} and Mg^{2+} make up more than 90 percent of all adsorbed cations. A healthy soil should have 40-50% Ca in its exchange complex.

Calcium ions are not strongly adsorbed by the soil colloids and thus the rate of leaching of Ca^{2+} is high. Sims and Ellis (1983) cited that under humid tropical climatic conditions, soils dominated by kaolinitic clays readily leaches out Ca^{2+}.

McLaughlin and Wimmer (1999) stated that as pH drops below 5.0, cationic Al species are dissolved more rapidly than Ca^{2+}, resulting in higher amount of Al species in the soil solution compared to Ca^{2+}. At pH levels above 5.0 causes the replacement of Ca^{2+} on soil cation exchange sites by H^{+} which in turn increases mineral weathering and thus availability of Ca^{2+} in soil solution.

Soils of the humid tropics are naturally acidic in reaction due to intense leaching and the subsequent loss of basic cations. The intensification of agriculture through high yielding crop varieties and large external inputs of acid producing chemical fertilizers, with scant attention paid to liming, aggravated the problem of soil acidity (KSPB, 2013).

Soils with low activity clays are inherently acid and contain very small amounts of exchangeable Ca and Mg. Aluminium toxicity and Ca and Mg deficiency occur in about 70% of the acid, infertile regions of the tropics (Varghese and Byju, 1993).

According to KSPB (2013) estimates, in Kerala, soil acidification has reached alarming proportion, impairing the productivity of most crop plants. Ninety percent of the soils of the Kerala suffer from acidity with fifty percent strongly to extremely acidic in reaction. The situation calls for immediate intervention to restore the soil productivity. Also forty percent of the Kerala soils are deficient in calcium.

Sureshkumar *et al.* (2013) also reported that the availability of calcium is very low in Kerala soils due to leaching under heavy rainfall. The reserve for the nutrient is also low in these soils. About 45 per cent of soils of Kerala are deficient in available calcium.

2.4.2. Role of calcium in crops

Plants growing with adequate Ca in their natural habitats have shoot Ca concentrations between 0.1 and 0.5% of dry weight. Ca is required for structural roles in the cell wall and membranes, as a counter-cation for inorganic and organic anions in the vacuole, and as an intracellular messenger in the cytosol (Marschner, 1995).

Calcium is required for cell elongation and cell division and plays a major role in the maintenance of membrane permeability (Fageria *et al.,* 1997). Cleland *et al*.(1990) reported that cell extension requires loosening of the cell wall, a process in which auxin induced acidification of the apoplast plays a role by replacing Ca^{2+} from the cross links of the peptic chain of the cell wall.

Caldwell and Haug (1982) cited that Ca^{2+} is adsorbed to negatively charged phosphate groups of membrane lipids and probably in this way that it restricts the permeability of membrane to hydrophilic solutes.

Cramer *et al.* (1985) pointed out that replacement of Ca^{2+} from the plasmamembrane by other cations like Na^{+} or cationic Al species has been a main factor involved in salinity stress. Calcium protects the plasma membrane from the deleterious effects of H^{+} ions at lower pH and also reduces harmful effects of Na^{+} in salt affected soils. It is also involved in the regulation of stomatal aperture (Epstein and Bloom, 2005).

Calcium as second messenger is involved in a large number of cellular functions that are regulated in plant cells by changes in cytosolic Ca^{2+} concentrations, such as ionic balance, gene expression, carbohydrate metabolism, mitosis and secretion (Bush, 1995). Calcium acts as a regulator ion in the translocation of carbohydrates through its effect on cells and cell walls (Bennett, 1993).Calcium is also reported to exert beneficial effect on plant vigor and stiffness of straw and also on grain and seed formation in cereals (Follett *et al.,* 1981).

Calcium promotes ion uptake and the formation of root mitochondria. Low supply of Ca inhibits nodulation, growth and nitrogen fixation of bacteria associated with the root of legumes (Pan, 2000). Fageria and Gheyi (1999) proposed that Ca is indispensible for the germination of pollen tube in plants from numerous families. Jha (2006) cited that after the discovery of calmodulin, it has become evident that calcium is not just a macronutrient but also a major controller of plant metabolism and development.

White and Broadley (2003) suggested that Ca flux to the xylem through the apoplastic pathway is markedly influenced by transpiration, which could lead to vagaries in the amount of Ca supplied to the shoot and development of Ca disorders. Ca deficiency occurs on soils with low base saturation and/or high levels of acidic deposition (McLaughlin and Wimmer, 1999).

Robinson (2010) stated that under Ca deficient situations banana exhibits interveinal chlorosis near the leaf margins and towards the leaf tip. When these patches die they create a serrated necrosis along the leaf edge. A temporary shortage of Ca causes the 'spike-leaf'symptom in the field, in which the lamina on new leaves is deformed or almost absent. Symptoms appear in early summer after a spring flush of growth and in plantations receiving a large amount of K. Sometimes only one leaf may be affected and leaf Ca levels are very low in such plants. Ca is very important in cell wall strength, thus in Ca-deficient plants fruit quality is inferior and the peel splits easily when ripe. Ca uptake by the plant depends not only on Ca concentration in the soil but also on the concentration of other elements, especially K and Mg.

In case of banana in flooded acid soils the excess of Fe caused impairment in the absorption and translocation of nutrients, particularly Ca and P, with 83 and 75% deficiency (Suresh, 2001). The author also reported calcium deficiency of 71.7% in the banana leaves grown in Fe toxic acid soil in high rainfall zone of Tamil Nadu.

2.4.3. Calcium uptake by crops

Generally Ca^{2+} concentration of the soil solution is about ten times higher than that of K^{+}, but its uptake rate is usually lower than K^{+}. Clarkson and Sanderson (1978) suggested that the low uptake of Ca^{2+} occurs since it can only be absorbed by young root tips in which cell walls of the endodermis are unsuberised.

Twyford and Walmnsley (1974) cited that in banana, the uptake of Ca during the course of plant growth follows dry matter accumulation at least until bunch emergence and during fruit growth further uptake of Ca depends upon the site.

Lahav and Turner (1989) and Lahav (1995) reported that altogether banana cv. Cavendish removed 227 kg of Ca/ha, and quantity removed in fresh fruit were 101kg/ha. For plantain it removed 158 kg of Ca/ha and quantity removed in fresh fruit was 9 kg. They also stated that proportion of Ca removed in fruit is 45 and 6% respectively for banana and plantain.

The uptake of calcium and magnesium increase at all stages of growth. Higher levels of nitrogen increased the uptake of Ca & Mg. The greatest respositories of calcium and magnesium were leaves, pseudostem and corm. Upto shooting these two elements continued to enter every part of the plant (Ragupathi *et al*., 2002).

The critical nutrient concentration of Ca in the leaf lamina of the third-youngest leaf of banana as reported by Stover and Simmonds (1987) is 0.45%.

Rufyikiri *et al.* (2000) reported that Al reduced Ca and Mg contents, increased K and P contents, and had no significant effect on N content. Although Al did not affect the rate of appearance of new leaves, it decreased total biomass,

pseudostem height, leaf surface area, growth of lateral roots, and number and diameter of root axes of banana.

2.4.4. Calcium and its effect on crop yield

Role of Ca as a soil conditioner has been widely recognized than as a plant nutrient.

Studies conducted by Nambiar *et al.* (1978) on the effect of graded doses of lime on growth and yield of banana var. Zanzibar at Banana Research Station, Kannara reported that application of lime contributed significantly for an increase in bunch weight, number of hands, and number of fingers per bunch.

Nutritional studies on banana var. Palayankodan carried out by Rajeevan (1985) at Kannara on the effects of split application of fertilizers, nutrient uptake and translocation reported that calcium were significant in the leaves and pseudostem. Calcium, magnesium, iron, manganese and zinc were found to be highest in leaves. Prema (1992) reported that banana yield was positively correlated with per cent calcium in leaf.

Moreno *et al.* (1999) who studied the effect of potassium, calcium and magnesium on banana yield (*Musa* AAA, Cavendish subgroup) reported that calcium application to banana significantly increased its yield.

Studies of Silva *et al.* (2008) on potassium, magnesium and limestone application in banana seedlings Prata Ana (AAB) reported that dry matter production of banana seedlings increased with increased application of limestone.

Study conducted by Yang *et al*. (2010a) in China reported that application of potassium, calcium, and magnesium promoted the growth of banana plant and offered a good material base for fruit, and it also increased the chlorophyll formation which in turn increased the photosynthetic rate.

Similar studies conducted by Yang *et al*. (2010b) on the effect of potassium, calcium and magnesium on yield, quality, and storage property of banana reported that that proper K, Ca and Mg application doses promoted banana growth and enhanced fruit yield and quality.

Crop responses to applied Ca (lime) have been spectacular in a number of field and green house studies ranging from 14.6 to 319 % (Singh and Behera, 2008). Most of the responses were in the range of 40 to 100 %.

Prochnow (2014) stated that a sound liming program improves soil physical, chemical and biological properties, symbiotic N fixation by legumes, positively influence the availability of plant nutrients and reduced toxicities to crops. He cited that worldwide the crop responses to applied Ca (lime) varied from 20 to 635%.

Sharma and Sarkar (2005) reported that conjunctive use of lime (0.2-0.4 t ha^{-1}) and recommended level of fertilizers on farmer's fields on acid soils has increased yields of a variety of crops by 49-189% over farmers' practice. Mathur (1994) showed that crop response to applied lime is due to Ca supply and not due to improvement in soil pH in groundnut and soybean. Application of Ca through gypsum increased the seed filling and vigour in sunflower in addition to seed yield (Ahmedkhan *et al*. 1990).

Liming have significant effect on the microbial population in the soils. The population of bacteria, actinomycetes and azotobacter increases in needbased liming treatments with continous manuring and cropping (Sharma *et al.,* 1983).

Tripathi and Shukla (2011) reported that in gooseberry the foliar application of calcium nitrate at 1.5% increased the weight, volume and pulp weight of the fruit and reduced the stone weight.

The field response of tannia to dolomite and gypsum as amendments indicated that dolomite as the most suitable for the acidic Ultisols of Kerala and hence standardised the rate of application as 1 t ha^{-1} (80 g/plant) as very effective in rectifying the problem with tannia cultivation and it also resulted in better vegetative growth and yield of the crop (John *et al.,* 2013).

In Uganda, banana soil receiving only household refuse, had significantly higher K, Ca, P and OM, and yield was 66% higher than that on soil without household refuse (Bananuka, 1994).

From 45 to 55% of the N, P, K and Ca in the whole banana plant is removed in the fruit, whereas for plantains a much lower proportion is removed in the fruit, especially for K and Ca. (Robinson, 2000).

In mango, although, calcium did not influence pollen germination and tube growth, it induced more fruit set in this mango cv. Namdokmai (Jutamanee *et al.,* 2002)

2.4.5. Calcium and crop quality

Calcium compounds extend the shelf-life of fruits by maintaining firmness, minimizing rate of respiration, protein breakdown, disintegration of tissues and disease incidence (Bangerth *et al.,* 1972).

In banana 4% $CaCl_2$ application delayed the onset of finger drop by 2 to 4 days, and hence an extension of shelf life of the fruit. The treatment also resulted in the lowest incidence of finger drop. Ripening of the fruit treated with calcium chloride proceeded normally and there was no effect on the physico-chemical and sensory attributes at the ripe stage (Esguerra *et al.,* 2009).

A review on calcium metabolism and its relationship with "maturity bronzing" in banana fruits by Cayon *et al*. (2007) stated that the incidence of maturity bronzing in dry season can be reduced by calcium nitrate fertilization.

Herrera *et al.* (2012) reported that in cape gooseberry when calcium was not applied, the cracking reached 38%, while with a dose of 100 kg ha^{-1}, the cracking was 27%.

In sweet cherry calcium treatment increased the chlorophyll a and chlorophyll b content. The mass of carotenoids were also in close connection with calcium sprays (Thurzo *et al.,* 2010).

Calcium spray trials on sweet cherry showed no significant results, in connection with fruit quality, contrary to copper-calcium spraying which resulted in higher cracking susceptibility and fruit firmness (Brown *et al.,* 1996).

Aguayo *et al*. (2006) reported the application of calcium at preharvest or postharvest stages can prevent postharvest disorder and fruit ripening. Calcium sprays to peach fruits as an aqueous solution has shown to be as ineffective as it has been proved in apple (Val *et al.,* 2008), as no effects on the fruit quality parameters, and on ion content in the peach skin or mesocarp have been recorded.

Poovarodom and Boonplang (2010) recommend that soil Ca together with pre-harvest spray of Ca + B is useful for reducing the number of translucent flesh disorder and gamboge disorder fruits in mangosteen.

Silva *et al.* (2006) reported that there were no significant effects of Ca treatments on plant growth, fruit weight, fruit size distribution or most indices of fruit quality of pineapple. There was also a significant negative correlation between translucency index and extractable soil calcium, basal white and green D-leaf calcium, and fruit calcium.

Fertilization with calcium increased fruit calcium levels and improved fruit storage life by reducing the incidence of internal browning associated with refrigerated storage (Herath *et al.,* 2000).

Tripathi and Shukla (2011) reported that in gooseberry the foliar application of calcium nitrate at 1.5% increased the TSS total sugar, ascorbic acid and reduced titratable acidity content of fruits as compared to the control.

2.5. MAGNESIUM

2.5.1. Magnesium in soils

Magnesium in soils occurs primarily in the form of ferromagnesian minerals like biotite, serpentine, hornblende, olivine and in secondary clay minerals like chlorite, vermiculite, and in relatively small concentrations in illite and smectites. Mg is also present in the form of carbonates (dolomite). In arid and semi arid regions Mg is also seen in the form of sulphates.

Depending upon the nature of mineral, degree of weathering, soil texture, and the prevailing climatic conditions, Mg content of soil types varies significantly. Generally Mg concentration of most soils lies in the range of 0.5 g/kg for sandy soils and 5 g/kg for clay soils. The total Mg content ranges from 0.1 per cent in coarse, humid regions to about 4 per cent in fine textured soils in arid and semi arid region with high Mg mineral.

Magnesium is likely to be deficient in acid, sandy, highly leached soils with low CEC, calcareous soils with inherently low Mg levels, acid soils receiving high rates of liming materials low in Mg, high rates of NH^{4+} or K^{+} fertilization, and crops with a high Mg demand. Coarse textured, humid region soils exhibit the greatest potential for Mg deficiency. Magnesium is prone to leaching and the leaching rates accounts for 2 to 30 kg Mg/ha/yr (Havlin *et al.,* 2012).

Magnesium has a large hydrated radius and sorbs weakly to soil colloids, predisposing it to leaching, particularly in acidic soil with low cation exchange capacity (Grzebisz, 2011). On alkaline soils, $MgCO_3$ formation and excess calcium (Ca), potassium (K) and sodium (Na) reduce Mg availability to crops (Broadley and White, 2010).

Mg toxicity can appear in certain locations, mostly in extreme serpentine (high magnesium:calcium) soils (Brady *et al.,* 2005) and in semi-arid regions, where water stress conditions can increase Mg metabolic pool in plants (Hawkesford *et al.,* 2012).

In acid soils, cationic Al species are believed to depress Mg uptake markedly (Grimme, 1983) while on calcareous soils the same effect is brought about by an excess of soluble Ca.

Soils having less than 4-15% of CEC occupied by Mg are considered to be deficient in Mg (Biswas *et al.,* 1985).

Loganathan (1973) studied the Mg status of south Indian soils and reported that the total Mg content as 0.22, 0.09, 0.11 and 0.03 per cent in the black, red, alluvial and lateritic soils respectively.

Prema (1992) stated that the total Mg reserves in Kerala are poor and thus Mg can be considered as a critical element in acid soils of Kerala, and in general, the soils of Kerala are deficient in total Mg reserves, with a mean value of 963.7ppm. Rani (2000) reported that many of the cultivated soils in Kerala are found to be deficient in available Mg and in many cases; crop growth is limited by Mg deficiency.

According to KSPB (2013) reports Mg is deficient in three fourth of the composite soil samples drawn from the state, pointing the widespread deficiency of the nutrient.

According to Sureshkumar *et al.* (2013) the availability of magnesium is very low in Kerala soils due to leaching under heavy rainfall. The reserve for the nutrient is also low in these soils, and about 80 per cent of soils of Kerala are deficient in available magnesium.

2.5.2. Role of magnesium in crops

The best known physiological roles for Mg are in harvesting solar energy by occupying the central position in the chlorophyll structure, as a cofactor and allosteric modulator for >300 enzymes (including carboxylases, phosphatases, kinases, RNA polymerases, and ATPases), and in chelation to nucleotidyl phosphate forms (Hawkesford *et al.,* 2012).

Magnesium occupies the central position of the chlorophyll structure and between 10 and 20% of the total Mg can be bound to that pigment (Wilkinson *et al.,*1990), with an even higher proportion upon Mg depletion (Hawkesford *et al.,* 2012). Magnesium is required for grana stacking (Kaftan *et al.,* 2002) and its depletion results in disorganized thylakoid membranes (Lu *et al.*, 1995).

Magnesium is also involved in the modulation of the activity of key photosynthetic enzymes, like ribulose-1,5-bisphosphate carboxylase/ oxygenase (Rubisco) that catalyzes the first major step of carbon. Low Mg levels affect the net rate of photosynthesis across a wide variety of plant species.

Mg is a primary constituent of chlorophyll and accounts for 15 to 20 per cent of the total Mg content of plants (Bybordi and Jasarat, 2010). Mg aids in phosphate metabolism, plant respiration, and the activation of several enzyme systems involved in energy metabolism (Fageria and Gheyi, 1999). According to Barber (1982) Mg is the most important cation species neutralizing the indiffusible anions of the thylakoid membrane.

Mg aids in the formation of sugars, oils and fats. It also activates formation of polypeptide chains from aminoacids (Tisdale *et al.,* 1985).

Jones and Huber (2007) reported that Mg is essential for the microbial growth in soil. He also stated that upto 90% or more of cellular Mg is bound mainly in ribosomes. It is associated with rapid growth, active mitosis, high

protein levels, carbohydrate metabolism, and oxidative phosphorylation in physiological young cells.

In all organisms, Mg is essential for structure and conformation of nucleic acids (Travers, 1989). Haeder and Mengel (1969) reported that when plants are Mg deficient the proportion of protein nitrogen decreases and that of non protein nitrogen increases. The effect is probably caused by the dissociation of the ribosomes into their subunits in the absence of Mg (Watson, 1965). Mg is required for effective release of organic acid anions from roots to modify an Al-toxic rhizosphere (Yang *et al.,* 2007).

Like Mg, Ca is also important in alleviating Al toxicity in acid soils. However, Mg can be protective against Al toxicity when added in micromolar levels, while Ca exerts its protective role in millimolar concentrations (Silva *et al.,* 2001).

Cakmak (1994) reported inhibition of root growth before any noticeable change in shoot growth and chlorophyll concentration. Consequently in Mg-deficient plants, the shoot: root ratio for both bean and wheat plants were increased. Magnesium-deficient leaves also contained elevated amounts of starch and reducing sugars, and severe inhibition in phloem export of sugars out of Mg-deficient leaves.

Fruit plants suffer from Mg deficiency in highly leached acid sandy soils, low soil pH, calcareous soils and through an imbalance of K/Mg and Ca/Mg ratios in the soil (Rajendran *et al.,* 2009).

Robinson (2010) reported that after N and K, deficiency of Mg is the most common in banana-growing countries. Symptoms include marginal yellowing on older leaves, changes in phyllotaxy, purple mottling of petioles and separation of leaf sheaths from the pseudostem, leaves turn yellow in a broad

marginal band, which consequently also reduces photosynthesis and yield potential.

A reduced yield caused by low Mg supply is proportional to reduced growth in plant parts of banana (Turner and Barkus, 1980).

Chalker and Turner (1969) reported that deficiencies usually occur where bananas have been grown for 10-20 years, without Mg fertilizer or where high amounts of K fertilizer have been used for a number of years (Messing, 1974). In banana, Mg toxicity is associated with a condition known as "deforestation blue" (Lahav and Turner, 1989).

In bananas high K/Mg ratios may induce typical mottling of the petiole, called ‘blue’ with a marked depressive effect on crop yields. Blue and other physiological disorders may appear with K/Mg ratio above 0.6-0.7. In acid ultisols of Cameroon K/Mg ratio over 0.7 induces blue only when root depth is poor (<20 cm), and when both exchangeable Mg level and total Mg reserve are below 1.3 and 45 meq 100^{-1} g respectively (Delvaux, 1989)

2.5.3. Magnesium uptake by crops

Shoot Mg concentration in plants adequately supplied with Mg generally approximate to 1 to 10 mg/g dry matter (Broadley *et al.,* 2004).

Mg concentrations required for optimal growth vary and are usually between 1.5 and 3.5 mg g^{-1} dry weight in vegetative tissues (Romeld, 2012). Mg concentrations below 1–2 mg g^{-1} leaf dry weight are frequently associated with the onset of chlorosis (Ding *et al.,* 2006).

The critical nutrient concentration in the leaf lamina of the third-youngest leaf of banana as reported by Stover and Simmonds (1987) is 0.2 to 0.22% magnesium. High levels of exchangeable Al, and an Al saturation of 65-70

percent reduced Mg uptake in many strongly acid soils often causes Mg deficiency (Havlin *et al.,* 2012)

Banana needs large quantities of nitrogen and potassium fertilization, which depressed magnesium and reduced the ability of plants to Mg uptake (Embleton and Jones, 1959; Westwood, 1978). In addition many Mg deficiency symptoms are being noticed on trees that receive heavy doses of potassium (Kassem and Seginy, 2002).

Lahav and Turner (1989) and Lahav (1995) reported that altogether banana removed 125 kg of Mg/ha, and quantity removed in fresh fruit were 49 kg/ha and for plantain 64 and 11 kg respectively. They also stated that proportion of Mg removed in fruit 39 and 17% respectively for banana and plantain.

Turner and Barkus (1983) reported in banana that there was no large overall effect of Mg supply on the uptake of elements other than Cu. High Mn supply reduced the uptake of Ca, Mg and Zn but had little effect on the other elements. K and Mn non-competitively inhibited Mg uptake and the K/(Ca + Mg) ratio of the lamina was unable to distinguish between K and Mg deficiency as it did not show an optimum relationship with yield.

Turner and Burkus (1980) reported that low Mg supply reduced the mean leaf area growth rate by 20% mainly by influencing the rate of leaf production. Low K and Mg supply reduced the number of live leaves present on the plants. Low Mg supply reduced dry matter production by 20%, the suckers present at ratoon harvest being affected most (41%) and the trash least (7%); fruit was 20% less.

Nutrient diagnosis study carried out in the black pepper gardens of Kerala and Karnataka revealed that 46% of the leaf samples had a Mg status below the required levels and the order of limiting nutrients were Mg > Cu > P=K=Zn > Mn (Hamza *et al.,* 2007).

2.5.4. Magnesium and its effect on crop yield

Johns and Vimpany (1999) reported that Mg deficiency occurred at low pH despite of regular foliar applications indicating the inefficacy of the foliar pathway for fertilizing bananas with macronutrients.

According to Prema (1992) in banana the magnesium treatments failed to influence the vegetative characters as well as yield and yield characteristics.

Significant effects were observed on days to flowering, plant height, number of leaves at flowering, leaf magnesium content, and yield in the highly weathered Puerto Rica soils and 25% yield increase was observed at the lowest magnesium increment relative to the control (Martinez *et al.,* 2002).

Mostafa *et al.* (2007) reported that Mg fertilization on banana cv. Grand naine showed positive effect on vegetative growth parameters, N, Mg, chlorophyll a and b content in leaves and it also improved bunch weight and fruit properties compared to that of control. In Williams banana, Mohammad (2007) found that application of Mg enhanced growth characters, hand weight, yield of crop compared to that of control.

Studies of Silva *et al.* (2008) on potassium, magnesium and limestone application in banana seedlings Prata Ana (AAB) reported that dry matter production of banana seedlings increased with increased application of potassium and limestone and decreased with increased magnesium application.

Study conducted by Yang *et al*. (2010a) in China reported that application of magnesium promoted the growth of banana plant and offered a good material base for fruit, and it also increased the chlorophyll formation which in turn increased the photosynthetic rate. Similar studies conducted by Yang *et al*. (2010b) on the effect of magnesium on yield, quality, and storage property of banana reported that proper Mg application doses promoted banana growth and enhanced fruit yield and quality.

Sztuder and Swierczewska (1998) found that, foliar application of magnesium to peas at 6 - 8 leaf stage and just before flowering stage and increased the number of branches per plant and seed yield was observed.

Muhammad *et al.* (2002) studied that, foliar application of magnesium as $MgSO_4$ on lentil Cv-Mazoor 93 recorded the highest number of pods per plant, number of seeds per pod and more seed weight of 1000 seeds were recorded.

Nannette (2011) suggested foliar spray of magnesium sulphate hydrate (epsomite) in tomatoes, peppers and roses for maximum growth, yield and quality of the produce. Magnesium treatments enhanced leaf total chlorophyll content, fruit set percentage, retained fruit percentage, yield, fruit physical and chemical properties and leaf minerals content in datepalm (Salama *et al.,* 2014).

Singh *et al.* (2008) reported that magnesium omission studies on sugarcane resulted in yield reduction upto 9.6 to 14.5 t/ha. Increase in efficiency due to magnesium application was found to be 3,687 to 3,713 kg cane/kg Mg with the net economic gain of Rs.10,560 to 16,830/ha.

2.5.5. Magnesium and crop quality

Gerendas and Fuhrs (2013) reviewed that increasing Mg supply on Mg-deficient sites tends to increase the quality of agricultural crops, particularly when the formation of quality traits is dependent on Mg-driven photosynthesis and assimilate translocation within the plant. They also pointed that Mg doses beyond those required for maximum yield rarely induce a further improvement of produce quality.

Due to the complex roles of Mg in chlorophyll and protein biosynthesis severe Mg deficiency results in interveinal chlorosis of older and fully mature leaves as Mg is highly mobile within the plant (Marschner, 2012). Negative impacts of an impaired primary (energy) metabolism are to be expected not only on crop yield (Grzebisz, 2013), but also on crop quality parameters.

Mohamed (2007) concluded that treating Williams banana plants six times with Mg, Zn and B either singly or in combinations had significant effect on improving fruit quality in terms of increasing fruit weight and dimensions, pulp weight and pulp / peel, total soluble solids percentage as well as total and reducing sugars and in decreasing peel weight percentage and total acidity percentage.

Mostafa *et al.* (2007) reported that Mg fertilization compared to control increased TSS percentage, total sugars, ascorbic acid content, and had no effect on acidity percentage in banana fruit cv. Grand naine.

In apple, Noe *at al.* (1995) reported that soil application of $MgSO_4$ increased the fruit firmness, texture, soluble solids, and juice pH while Marcelle (1995) reported negative effect on fruit firmness, texture, soluble solids, dry matter and positive effect on juice pH by the application of N, P, K, Ca, and Mg.

In pine apple soil application of MgO increased the average fruit length, fruit width and core diameter (Velez and Borges, 1995).

Vrataric *et al.* (2006) reported that foliar application of $MgSO_4$ increased the oil and protein concentration in soyabean. Jahangir *et al.* (2005) also reported the increase in oil concentration of Indian mustard following soil application of $MgCO_3$ and $MgSO_4$.

Increase in concentration of aminoacids, polyphenols, and catechin in tea as a result of soil application of $MgSO_4$ was given by Jayaganesh and Venkatesan (2010). Han *et al.* (2010) studied the effect of $MgSO_4$ and Mg-EDTA and found increased levels of chlorophyll and soluble protein content in cabbage. Sachray *et al.* (2002) reported improved colour characteristics of aster by the application of $MgCl_2$ and $MgNO_3$ solutions.

2.6. SULPHUR

2.6.1. Sulphur in soils

Sulphur occurs in the soil both in organic and inorganic forms but in most soils organically bound S provides the major S reservoir. In most instances in soils of humid and semi humid areas the range of total S is from 100 to 1000 mg S/kg, a range that is similar to that of total P. Organic S concentration generally decreases with soil depth and approaches zero in soil depth more than 1.5m. In peat soils, organic sulphur may amount to 100% of total S. Inorganic forms of S in soil consists mainly of sulphates like $CaSO_4$, $MgSO_4$ and Na_2SO_4.

Under waterlogged conditions, inorganic S occurs in reduced forms such as FeS, FeS_2 and H_2S. Total soil levels depend on organic matter contents and also on the climatic conditions. In soils under humid regions high amount of SO_4^{2-} are leached whereas in arid soils SO_4^{2-} accumulates in the upper profile.

Most of the S in soils is bound to organic molecules, making up more than 90% of the S present in soil (Solomon *et al.,* 2009). Inorganic S generally accounts for <10% of total S (Solomon *et al.*, 2009). Despite the fact that plants absorb S mainly as sulfate (SO_4^{2-}), organic S (S directly bonded to C and ester S) pool is an important source of S to plants during their growing season (Bona & Monteiro, 2010).

The total soil S content ranges from 0 to 600 mg kg^{-1} in arable land around the world (Zhao *et al.,* 1996) and up to 5000 mg kg^{-1} is found in soil with high organic matter content (Freney *et al.*, 1991). Barber (1979) reported that 3% of the organic matter content of Indian soils was mineralized at a rate of 2.4% per year. Assuming that the mineralized organic matter contained 0.5% sulfur, this would release about 3.5mgS/kg of soil or 7 kg S/ha annually.

Nambiar (1988) reported that increase in S deficient soils in India is due to the use of high-analysis, S-free fertilizers in combination with reductions in atmospheric S from pollution. Kumar and Sidhu (2013) also reported the

depletion of soil S was caused by widespread use of modern high yielding varieties, high analysis fertilizers without micronutrients, and intensive cropping.

Most of the Indian soils have low total reserves of sulphur because of low quantities of organic matter and its rapid mineralization as well as leaching losses (Ganeshamurthy and Saha, 1999). The total S content varies from 19 to 9750 mg/kg (Tandon, 1989). But most of the cultivated soils contain total S in the range of 50-300 mg/kg. Very high values of total S in excess of 1000 mg/kg are generally encountered in problematic soils such as saline and acid-sulphate soils (Takkar *et al.,* 1997).

In 1991 about 130 districts were found to be deficient in sulphur as reported by Singh (2006) while Tewatia *et al.* (2006) reported that 46 percent of Indian soils are deficient in available S as on 2006.

Indian soils exhibit wide degree of S deficiency because of the use of S-free fertilizers, increased S removal following intensification of agriculture, removal of S by grain and straw/stover from the fields, low level of fertilizer use in pulses and oilseeds growing areas, progressive negative balance of S in the cropping systems, reduction in environmental addition of S, declining native S reserves, minimal use of S-containing pesticides, leaching losses of S following flood irrigation, some areas due to heavy rains (Sahrawat *et al.,* 2007*;* Hegde and Babu, 2009).

Kanwar and Takkar (1964) proposed 10.0 mg kg^{-1} as the upper limit for soil S characterization. Generally to categorize the S status of soils in different agroecological regions for recommendations in the soil testing laboratories the critical limits are < 7.5 mg kg^{-1} – low, 7.5-15.0 mg kg^{-1} – medium, > 15.0 mg kg^{-1} – high. According to KSPB (2013) estimates deficiency of sulphur in soils of Kerala is to a tune of 30 percent.

2.6.2. Role of sulphur in crops

Sulphur is best known for its role in the formation of aminoacids-methionine (21% S) and cysteine (27% S), synthesis of proteins and chlorophyll, oil content of the seeds and nutritive quality of forages (Jamal, 2009).

The range of biological compounds that contain S is vast. S is found in vitamins viz, biotin and thiamine; cofactor S- adenosyl-L-methionine, coenzyme A, molybdenum cofactor, and lipoic acid, the chloroplast lipid sufloquinovosyl diacylglycerol, and many secondary compounds (Leustek, 2002).

Jamal *et al.* (2010) reported that S is specifically involved in nitrogen fixation in legumes and S additions significantly increased N_2 fixation, nodule weight plant^{-1}, nodule weight per unit weight of root and N_2-fixation per unit weight nodule.

Organic sulfates may serve to enhance water solubility of organic compounds, which may be important in dealing with salinity stress (Clarkson and Hanson, 1980). Friedrich *et al.* (1977) observed severe reduction in nitrate reductase activity (NRA) in S-deprived maize seedlings. Fertilization with soil applied S in sulfate form decreases fungal diseases in many crops (Haneklaus *et al.,* 2007).

Fageria and Gheyi (1999) summarized role of S in various physiological activities like protein formation, enzyme activation, nodule formation, chlorophyll formation, seed and fruit maturity, nitrogenase formation, oxidation-reduction reactions, besides it increases oil content in oilseeds, winter hardiness, drought tolerance, milling and baking qualities and synthesis of vitamins, hormones, glutathione, glucosides and sulfolipids.

Walmsley and Twyford (1976a) reported that next to potassium, sulphur is considered as the fourth important nutrient, as bananas require 17 kg/ha/year.

2.6.3. Sulphur uptake

Absorption of sulphur and its uptake by plants from soils depends upon the nature of crop, soil type, available sulphur and other nutrients status and crop productivity.

Ganeshamurthy and Satisha (2012) stated that those crops having high nitrogen needs also have a high sulphur need due to their mutual functions in plant growth, such as protein formation. Many vegetable crops, spices, palms and fruits require sulphur for achieving high yield as well as quality.

Sulphur uptake in most crops ranges from 5 to 40 kg/ha. Usually, sulphur requirements of crops ranges from 9-15% of N uptake and may approach 25-30% in oilseeds (Singh, 2001). Crops differ widely in their S requirement, with plant dry matter concentration typically between 0.1 and 1% S. The S requirement is typically greatest for brassicas, followed by legumes, and then by cereal grasses (Norton *et al.,* 2013).

Critical limits (0.01*M* $CaCl_2$-S) being followed for various crops are - mustard 9 mg kg^{-1}, groundnut 10.0 mg kg^{-1}, soybean 11.3 mg kg^{-1} (Ganeshamurthy, 1996), wheat 15 mg kg^{-1}, rice 10.5 mg kg^{-1}, and sunflower 19.0 mg kg^{-1}.

Ganeshamurthy and Sathisha (2012) reported that among fruit crops banana and pineapple removes significantly higher quantity of sulphur compared to any other fruit crops. For a banana crop producing an economic yield 100t/ha removes 14.4kgS/ha.

The application of sulphur and micronutrients increased the leaf N, K and S contents in banana (Pertiz and Das, 1998; Mostafa and Kader, 2006).

According to Ragupathi *et al*. (2002) in banana, sulphur is evenly distributed in the plant while bulk of Fe was in root or stem, while sizeable amount of Mn, Zn and Cu was found mobilized to leaves.

Robinson (2010) stated that in banana deficiencies of S have been reported in the field. S is actively distributed from old to young leaves and, with a shortage; symptoms appear in the young leaves which become yellowish-white. As the deficiency progresses, necrotic patches appear on leaf margins and vein thickening occurs. Growth is stunted and the bunch is small or choked. The most rapid uptake of S occurs between sucker selection and flowering. After this, uptake is reduced and S needed for fruit growth comes from leaves and pseudostem.

Ganeshamurthy and Sathisha (2012) reported that optimum sulphur standard for Elakki banana as 0.06-0.13%. They explained that in case of S deficiency young leaves of banana show chlorosis, and if deficiency continues, chlorosis becomes more clear and pale stripes became visible between the veins. Besides, growth is retarded, edges of young leaves turn yellowish white and necrotic patches appear on older leaves. Youngest uncurled leaves become yellowish white. Such plants do not flower and plant may die. In moderate deficiency, only small fruits are produced.

2.6.4. Sulphur and its effect on crop yield

Deficiency of S is reported on all types of soils and in most of the regions (Scherer, 2001). Supplying agricultural crops with adequate S is essential in order to maximize production (Hall and Solberg, 2001).

Response of crops to applied S depends on soil type, S status of the soil, soil moisture, source of S and crop requirements. Significant response in terms of seed yield and oil content in oilseed crops has been reported due to S application (Hegde and Babu, 2009).

Espinosa and Belalcazar (2000) reported that in plantains, highest fruit yields in high density planting occurred only when N, K and S were applied together.

Effect of micro granular sulphur on nutrient uptake, soil properties and yield of banana was studied by Chaure *et al.,* (2009) found out that two split application of micro granular sulphur significantly increased the number of hands per bunch, bunch weight and hence the yield.

Kumar and Kumar (2008) studied the efficacy of sulphate of potash on banana cv. Robusta (Cavendish- AAA) and recommended to include SOP in banana nutrition for getting maximum bunch weight.

Kumar and Kumar (2007) reported that 1.5% SOP spray in banana cv. Neypoovan was most profitable, improved the final fruit yield and doubled the net income compared to control.

Mostafa and Kader (2006) studied the sulphur fertilization effects on banana cv. Grand nain and concluded that addition of sulfur at any dose resulted in greater bunch weight and number of fingers than the control.

Kavitha and Menon (2013) reported that combined application of sulphur and magnesium resulted in higher rhizhome yield in Kacholam.

The average response of rice and wheat to applied S was 17% in rice and 25% in wheat. Application of S to sugarcane increased the yield of plant and ratoon cane by 9.9 and 13.7%, respectively, on alluvial soils of Lucknow (Shukla and Menhilal, 2007).

In rice sulphur application at the rate of 40 kg/ha resulted in significant response in rice and it produced maximum grain and straw yield (Kumar *et al.,* 2011).

Singh *et al.* (2008) reported that sulphur omission studies on sugarcane resulted in yield reduction upto 8.8 to 11.8 t/ha. Increase in efficiency due to sulphur application was found to be 1,615 to 1,857 kg cane/ kg S with the net economic gain of Rs.9,680 to 12,100/ ha.

Aulakh (2003) reported that S requirement is highest in case of oilseeds followed by pulses, forages, tuber crops and cereals. In blackgram S application at the rate of 40 kg/ha produced maximum plant height, number of leaves/plant, number of nodules/plant, haulm yield, grain yield, protein content, P, S content (Mir *et al*., 2013).

2.6.5. Sulphur and crop quality

Crops grown in S-deficient soils can suffer reduced yields as well as poor product quality. An adequate S supply is a major factor in supporting plant protein quality, where it plays a major factor in the structure and function of enzymes and proteins in leafy tissues and seeds.

Biswas and Tewatia (1991) indicated that most crop species have higher yield and better quality products when there is an ample amount of S available in the soil.

S is reportedly critical to overall plant nutrition of crops because deficiencies in S can result in a reduced ability to locate and utilize various micronutrients (Panchuck *et al.,* 2000).

In banana, fruit quality is mainly judged by the sugar content and acidity in the pulp. The foliar SOP sprays appeared to be effective in enhancing various quality parameters such as TSS, reducing, non-reducing and total sugars and acidity in banana cv. Neypoovan (Kumar and Kumar, 2007).

Kumar and Kumar (2008) reported that SOP application improved the sugar content and vitamin C content in banana cv. Robusta. Mostafa and Kader (2006) found that in banana cv. Grand nain, fruit total soluble solids, total sugars and vitamin C content were significantly increased with increasing S-application rates compared with the control.

Sulphur uptake was positively and significantly correlated with the ascorbic acid content of fruits in okra (Rani and Jose, 2009). Ganeshamurthy (1996) showed that application of S to soybean increased the S content of seeds and the oil yield. External application of S to rice in soils of Andaman and Nicobar increased the S content of rice (Ganeshamurthy *et al.,* 1995). Increased S content following application of S in food legumes has also been reported by Srinivasarao *et al.* (2004).

Application of S at the rate of 60 kg/ha significantly increased the seed yield, oil content of mustard and it also increased the iodine number, acid value of the oil (Hegde and Babu, 2009). Adequate S nutrition is essential for the catabolism of the amino acids methionine and cysteine (Messick *et al.,* 1991), and is necessary for ascorbic acid retention in the tissues of cruciferous vegetables (Albrecht *et al.,* 1990).

Kavitha and Menon (2013) reported that sulphur application increased the oleoresin and essential oil content in Kacholam.

An adequate supply of cysteine plays a central role in giving cereal proteins their shape and functional properties. Because of this, bread baked with low-S wheat will not rise, and results in dense and poorly shaped loaves (Norton *et al.,* 2013).

2.7. BORON

2.7.1. Boron in soils

Boron is the only non-metal among the micronutrients and occurs in low concentrations in the earth's crust and in most igneous rocks (10 mg/kg).

Boron is unique among the essential micronutrients as it is the only element present in soil solution as a non ionized space over the pH range suitable for the plant growth (Arora and Chahal, 2007).

Soil B exists in rocks and minerals, adsorbed on clay surface and Fe/Al oxides, combined with OM, and in soil solution. The main B mineral in soils is tourmaline. Total B concentration in soils varies between 2 and 200 ppm and frequently ranges from 7 to 80 ppm. The available, hot water soluble fraction in soils adequately supplied with B ranges from 0.5 to 2 mg B/L (Sillanpaa, 1982).

Boric acid (H_3BO_3) is the main constituents of soluble boron in soil, and this acid does not dissociate under most prevailing soil pH conditions (pH 4-8) and thus, in contrast to all other essential plant nutrients, boron is mainly present in a non ionized form in soil solution (Keren and Bingham, 1985). So B can be leached so easily from the soil.

Gupta and Cutcliffe (1978) reported that more than 60% of applied B was not recovered in the upper layer of a podzolic soil five months after application. However, in soils of arid and semi arid regions, B may accumulate to toxic concentrations in the upper soil layer because of lack of drainage (Keren and Bingham, 1985).

The availability of boron is greatly influenced by soil characteristics like pH, EC, organic matter, texture and $CaCO_3$ content. Barber (1995) reported that B adsorption by fine textured soils is 2-3 times greater than by coarse textured

soils. Boron deficiency is frequently encountered on low organic matter, sandy soils. High rainfall and leaching losses reduce B availability. Dry weather can also trigger a deficiency (Fageria and Gheyi, 1999) which can be attributed partly to the reduced number of microorganisms that can release B from the parent materials (Gupta, 1979).

The total B content of Kerala soils ranges from 27.5 to 330 mg/kg. About 65% soils of Kerala are deficient in available boron. The soils of southern and northern coastal plains, low lands of lateritic origin are deficient in boron. Very high levels of available boron (>5mg/kg) as well as boron retention are detected from Pokkali and Kaipad tracts due to sea water inundation and high levels of organic matter (George, 2011; Santhosh, 2013).

Santhosh (2013) reported that the relative dominance of B fractions is in the order of residual B (RS-B)> oxide bound B (OX-B)> organically adsorbed B (OR-B) > specifically adsorbed B (SA B) > readily soluble B (RS B). B adsorption by plants is mainly from RS-B, OR-B, and SA-B fractions. The level of readily soluble B (easily available fraction) fraction is maintained by SA-B as well as OR-B as and when it is depleted.

Sureshkumar *et al.* (2013) stated that at low pH, most of the boron compounds are soluble and thus boron remains available to plants as boric acid. In coarse textured soils with low organic matter (sandy and lateritic soils of Kerala), having low pH, as the boron retention capacity is low, boron is lost by leaching. Availability of B in acid soils decreases with increase in soil pH. Liming of soil reduces boron availability temporarily due to lime induced boron adsorption and occlusion as $B(OH)^{4-}$ by freshly precipitated Al and Fe hydrous oxides. Organic matter can adsorb and retain B in both in acidic and alkaline pH since mineralization of soil organic matter releases boron and makes it available to plants.

The soil can be classified as low (< 0.5 mg B kg-1), medium (0.5-2.0 mg B kg-1) and high (>2.0 mg B kg1) as per categorization given by Tandon (1992). Boron deficiency has been reported in 132 crops in 80 countries (Shorrocks, 1997) and is a major cause of yield loss in China, India, Nepal and Bangladesh (Anantawiroon *et al.,* 1997). According to KSPB (2013) estimates 59 percent of the Kerala soils are deficient in Boron which suggested that boron deficiency is significant and extensive, requiring immediate intervention.

2.7.2. Role of boron in crops

Boron is essential micronutrients for plants, but at the same time, its range between deficiency and toxicity is narrower than that of any other element (Goldberg, 1997).

Boron helps in sugar translocation as it forms complexes with polyhydroxyl compounds including sugar and alcohol that easily traverse through cellular membranes. It is important for pollen tube growth and hence fruit set. Its role has also been implicated in RNA and IAA metabolism (Marschner, 1995).

In plants, B is necessary for the following important processes: (1) early growth and flower bud formation (Kamali and Childers, 1970); (2) production of pollen grain (Argawala *et al.,* 1981), pollen germination and pollen tube growth (Dickinson, 1978); (3) cell division and differentiation and root tip development (Brown and Hu, 1996); (4) metabolism of hormones, auxin regulation, and movement of the natural hormones to encourage both cell division and cell enlargement (Nijjar, 1985); (5) translocation of calcium (Wojcik and Wojcik, 2003); (6) carbohydrates transport (Marshner, 1986); (7) increasing fruit set (Hanson, 1991) and improving yields (Chaplin *et al.,* 1977).

Boron performs a physical role in maintaining cell-wall extensibility. Formation of borate esters with hydroxyl groups of cell-wall carbohydrates and/or glycoproteins has been proposed as a mechanism for cross linking cell-wall polymers. This type of bonding is accountable for brittle leaves of boron-deficient plants, whereas plants grown with supra-optimal levels of boron produce leaves that are plastic or elastic in their response to bending (Loomis *et al.,* 1992).

Boron plays an important role in maintaining plasma membrane integrity, possibly by linking glycoprotein and glycolipid components of the plasma membrane bilayer through its ability to complex OH-containing polysaccharides (Parr *et al.,* 1983) or through its involvement in enzyme systems such as ATPases (Goldbach, *et al.,* 1991) or esterases (Shivanna and Harrison, 1981) that become active on pollen hydration.

Boron treatment of low boron plants stimulates ATPase activity, NADH oxidase activity and ion transport (Barr *et al.,* 1993).

Shkolnik (1984) observed that several enzymes, normally bound to membranes or walls in a latent form, become active when released under boron deficient conditions. These enzymes include ribonuclease, glucose-6-phosphate dehydrogenase, phenylalanine ammonia lyase, β-glucosidase and polyphenoloxidase. Release of these enzymes under boron-insufficient conditions could severely alter plant metabolism, deplete RNA and increase phenolic synthesis. Many of the phenolics are potent growth inhibitors (Leopold and Kreidemann, 1975) the same phenolics also inhibit ion uptake and thus retard membrane function (Glass and Dunlop, 1974).

Visser (1955) showed that continuous and ample supply of boron was required for pollen tube growth and speculated that boron was complexing with cellular materials during the tube elongation process. Johri and Vasil (1961)

demonstrated that boron was more critical for pollen tube elongation than for pollen germination.

Soil boron application increased the activity of catalase and glutathione reductace, which act as antioxidants thus saving the electron transport mechanism of the plant from getting oxidized by free radicles like superoxide radicles, singlet oxygen radicles, etc (Wojcik *et al.,* 2008).

Robinson and Sauco (2010) stated that in banana, B deficiency has been reported on some acid soils in tropical America, and is also prevalent in the subtropics on low pH soils. Symptoms include leaf tip curling and deformation, prominent raised leaf veins, bent cigar leaf and, short white stripes perpendicular to the veins on the underside of the lamina.

Boron deficient rubber trees have thinner middle lamella due to low supply of calcium pectate which promotes the adhesion between cells (Moraes *et al.,* 2002). Symptoms of B deficiency involve tissue brittleness, but excess B produces unusually resilient cell wall which could support the high turgor pressure (Loomis and Durst, 1992).

Lahav and Turner (1983) reported that critical B concentration in lamina of third leaf of banana cv. Cavendish as 11 ppm. Range of norms given for B by the Agricultural Research Council of Institute for Tropical and Subtropical Crops lies between 15 to 60 ppm.

Moreira and Almeida (2005) reported that B deficiency causes severe growth inhibition of the banana plant, with negative effect on the pulp consistency of the fruits.

Boron toxicity studies carried out by Vargas *et al.* (2007) in Costa Rica stated that continuous necrosis developed from an irregular chlorotic area, from the edge towards the internal part of the leaf blade, in banana plants (*Musa*

AAA, cultivars Grande Naine and Valery) while the central portion of the leaf remained green. Soil and foliar analyses showed that symptoms were caused by high boron concentrations, probably due to excessive soil or foliage applications of the nutrient, or to the effect of very frequent applications of boron during fertigation, combined with a decrease of calcium in the leaf.

2.7.4. Boron and its effect on crop yield

Spectacular response of cereals, pulses, oilseeds and cash crops to B application (0.5 to 2.5 kg ha^{-1}) had largely been observed on B deficient soils of Bihar, Orissa, West Bengal, Punjab and Assam (Sakal and Singh, 1995).

Moreira *et al.* (2011) showed that in Xanthic Ferralsol, the application of 3.4 kg B ha^{-1} in the first cycle and 1.3 kg B ha^{-1} in the second cycle, respectively, guarantees an adequate B status in banana plants (10 to 25 mg kg^{-1}), with a maximum yield achieved by addition of 4.1 kg B ha^{-1} in the first cycle and 6.3 kg B ha^{-1} in the second cycle.

Similar studies conducted by Castro *et al*. (2010) on efficiency of boron application in an oxisol cultivated with banana in the Central Amazon found out that in dystrophic yellow latosol – oxisol (xanthic ferralsol) in the moist tropical Amazon, in the first two cycles, the B rates of 4.1 and 6.3 kg ha^{-1} led to the greatest estimated banana yield.

According to the studies of Nomura *et al.* (2011) on boron application in banana plant reported that B application did not influenced the development and production of banana plant 'Grande Naine'.

Wojcik *et al.* (2008) observed that pre-bloom foliar application of boron to apple trees increases fruit set as well as yield.

In pear tree increasing B concentration enhanced fruit set, fruit retention and yield, measured either as number of fruits or total yield and reduced the vegetative growth parameters (Shazly and Kotb, 2011).

Foliar application of 100 ppm of boric acid three times viz., 40, 50, 60 days after sowing produced significant improvement in growth parameters of tomato (Bhatt *et al.,* 2004)

In almonds, fruit set, fruit number and cumulative yields were increased in response to B fertilization. Kernel production was approximately 21-24% higher in the B treated plants than that of control. Cumulative fruit number per tree was also significantly enhanced by B treatments (Rufat and Arbones, 2006).

Shukla *et al.* (2011) reported that borax concentrations 0.4% as most effective in increasing the length, diameter, weight and volume of the fruit in aonla.

2.7.5. Boron and crop quality

Studies conducted by Castro *et al*. (2010) on efficiency of boron application in an oxisol cultivated with banana found out that B application increased the pulp/peel ratio, the pulp texture and the total B concentrations in the leaves and fruits.

Boron sprays at blooming time had a positive effect on photosynthetic pigment and carotenoid contents of sweet cherry leaves, but two-times spraying had a negative effect on them (Thurzo *et al.,* 2010).

Shazly and Kotb (2011) reported that B application increased TSS and total sugars and reduced acidity in pear trees. Wojcik *et al.* (2008) also reported an increase in total soluble solids as well as total acidity in apples due to soil boron application.

Xuan *et al.* (2003) concluded that pre-harvest boron application improved the ability of fruit tissue to better resist impaired storage conditions with the result of avoiding typical browning disorders.

In aonla, borax concentration of 0.4% was found to be effective in increasing the TSS, total sugar, ascorbic acid content, and reduced titrable acidity (Shukla *et al.,* 2011).

Materials and methods

1. MATERIALS AND METHODS

An investigation was carried out at College of Agriculture (COA), Padannakkad and Regional Agricultural Research Station farm of Nileshwar during December 2013 to January 2015. The objective was to study the effect of application of calcium, magnesium, sulphur and boron in potting mixture for hardening and application of these nutrients in the field conditions of tissue culture banana var. Nendran. The whole study was conducted in two steps.

Part I: Studies on hardening of TC plants using fortified potting mixture

Part II: Field Experiment

3.1 POT CULTURE EXPERIMENT

The hardening studies were conducted in the mist chamber of COA, Padannakkad with tissue culture raised plants of uniform size derived from same lot. The experiment was carried out in completely randomized design with two levels each of calcium, magnesium, sulphur and boron. There were 16 treatment combinations and one control with three replications each. Four plants were maintained in each of the replications, so that the total number of plants being 204. The potting mixture was prepared uniformly with 1:1:1 ratio of soil, sand, cowdung and then it was fortified using the various treatment combinations of calcium, magnesium, sulphur and the plants were raised in these polybags for a period of one month in mist chamber (primary hardening) followed by secondary hardening for another one month under partial shade conditions. Boron applications in the form of foliar spray were given out immediately after planting and one month after planting.

3.1.1. Design and layout

Crop : Tissue culture banana
Variety : Nendran
Design : CRD

Treatments : 17

Replications : 3

Treatments were combinations of two levels each of Ca, Mg, S and B plus one control (2 x 2 x 2 x 2 *ie* 16 plus one control).

3.1.2. Levels of Nutrients

Ca - 75 and 150 ppm calcium as $Ca(OH)_2$

Mg - 25 ppm and 50 ppm magnesium as MgO

S - 25 ppm and 50 ppm sulphur as K_2SO_4

B - 0.25% and 0.5 % boron as borax foliar

The different treatment combination were as listed below.

3.1.3. Treatment combinations

T_1 - 75ppm Ca + 25ppm Mg + 25ppm S + 0.25% B

T_2 - 75ppm Ca + 25ppm Mg + 25ppm S + 0.5% B

T_3 - 75ppm Ca + 25ppm Mg + 50ppm S + 0.25% B

T_4 - 75ppm Ca + 25ppm Mg + 50ppm S + 0.5% B

T_5 - 75ppm Ca + 50ppm Mg + 25ppm S + 0.25% B

T_6 - 75ppm Ca + 50ppm Mg + 25ppm S + 0.5% B

T_7 - 75ppm Ca + 50ppm Mg + 50ppm S + 0.25% B

T_8 - 75ppm Ca + 50ppm Mg + 50ppm S + 0.5% B

T_9 - 150ppm Ca + 25ppm Mg + 25ppm S + 0.25% B

T_{10} - 150ppm Ca + 25ppm Mg + 25ppm S + 0.5% B

T_{11} - 150ppm Ca + 25ppm Mg + 50ppm S + 0.25% B

T_{12} - 150ppm Ca + 25ppm Mg + 50ppm S + 0.5% B

T_{13} - 150ppm Ca + 50ppm Mg + 25ppm S + 0.25% B

T_{14} - 150ppm Ca + 50ppm Mg + 25ppm S + 0.5% B

T_{15} - 150ppm Ca + 50ppm Mg + 50ppm S + 0.25% B

T_{16} - 150ppm Ca + 50ppm Mg + 50ppm S + 0.5% B

T_{17} – control (*i.e.* only potting mixture)

3.1.4. Biometric observations

The important biometric observations like plant height, leaf number, leaf length, leaf breadth were taken at the end of two months of treatment imposition. Plant height was measured from the base of the peudostem in the poly bag to the youngest leaf. Leaf length was measured from petiole to tip and leaf breadth at its widest middle portion. The number of photosynthetically active leaves was also recorded.

3.2. FIELD EXPERIMENT

The field experiment was carried out at Regional Agricultural Research Station farm of Pilicode at Nileshwar, Kasaragod, Kerala to study the effect of application of calcium, magnesium, sulphur and boron in tissue culture banana var. Nendran.

Two hundred and four hardened tissue culture banana plantlets were planted in randomized block design with seventeen treatments and three replications such that four plants were maintained in each plot. Nitrogen, phosphorus, potassium application and other cultural practices were followed as per POP, KAU (2011) uniformly for all the treatments.

3.2.1. Design and layout

Variety : Nendran
Design : RBD
Treatments : 17
Replications : 3
Number of plants per plot : 4

Treatments were combinations of two levels each of Ca, Mg, S and B plus one control (2 x 2 x 2 x 2 *ie* 16 plus one control). Altogether there were 17 treatments and 3 replications and in each plot there were 4 plants.

3.2.2. Levels of Nutrients

Ca - 75 and 150 g calcium / plant as $Ca(OH)_2$
Mg - 25 and 50 g magnesium / plant as MgO
S - 25 and 50 g sulphur /plant as K_2SO_4
B - 0.25% and 0.5 % boron as borax foliar with uniform wetting of all leaves

Calcium, magnesium and sulphur were applied on second and fourth month after planting. Boron as borax foliar spray were given periodically at first, second and fourth month after planting and an ultimate spray after the bunch emergence.

3.2.3. Treatment combinations

T_1 - 75g Ca + 25g Mg + 25g S + 0.25% B
T_2 - 75g Ca + 25g Mg + 25g S + 0.5% B
T_3 - 75g Ca + 25g Mg + 50g S + 0.25% B
T_4 - 75g Ca + 25g Mg + 50g S + 0.5% B
T_5 - 75g Ca + 50g Mg + 25g S + 0.25% B
T_6 - 75g Ca + 50g Mg + 25g S + 0.5% B
T_7 - 75g Ca + 50g Mg + 50g S + 0.25% B
T_8 - 75g Ca + 50g Mg + 50g S + 0.5% B
T_9 - 150g Ca + 25g Mg + 25g S + 0.25% B
T_{10} – 150g Ca + 25g Mg + 25g S + 0.5% B
T_{11} – 150g Ca + 25g Mg + 50g S + 0.25% B
T_{12} – 150g Ca + 25g Mg + 50g S + 0.5% B
T_{13} – 150g Ca + 50g Mg + 25g S + 0.25% B
T_{14} – 150g Ca + 50g Mg + 25g S + 0.5% B
T_{15} – 150g Ca + 50g Mg + 50g S + 0.25% B
T_{16} – 150g Ca + 50g Mg + 50g S + 0.5% B
T_{17} – control (as per POP recommendations only)

The whole experiment was layed out as RBD and it is as shown in figure 1.

Fig 1: Layout of field experiment

Block 1	**Block 2**	**Block 3**
T_8	**T_{14}**	**T_{12}**
T_7	**T_4**	**T_8**
T_{16}	**T_3**	**T_{17}**
T_{17}	**T_1**	**T_{16}**
T_6	**T_9**	**T_4**
T_{12}	**T_{15}**	**T_1**
T_{10}	**T_8**	**T_{15}**
T_{13}	**T_{17}**	**T_{10}**
T_4	**T_{12}**	**T_6**
T_2	**T_{16}**	**T_9**
T_3	**T_2**	**T_5**
T_{15}	**T_{10}**	**T_2**
T_1	**T_7**	**T_{13}**
T_9	**T_6**	**T_3**
T_{14}	**T_{11}**	**T_{11}**
T_{11}	**T_5**	**T_7**
T_5	**T_{13}**	**T_{14}**

3.2.4. Biometric observations

Biometric observations like pseudostem girth, plant height at the time of shooting, leaf number and number of suckers at the time of harvest, weight of male bud, bunch weight, number of hands, number of fingers, weight of fingers, length of fingers, breadth of fingers, days to bunch emergence, days to harvest and incidence of pest and disease were recorded.

3.2.4.1. Plant height

Plant height at the time of shooting was recorded from the ground level upto the peduncle and expressed in meter.

3.2.4.2. Pseudostem girth

Girth of pseudostem at 30cm height from the ground level at the time of bunch emergence was recorded.

3.2.4.3. Leaf number

Number of leaves at the time of bunching was recorded.

3.2.4.4. Number of suckers

Number of suckers remaining at the base of the crop at the time of harvest was recorded.

3.2.4.5. Weight of male bud

Fresh weight of male bud one month after bunch emergence was recorded.

3.2.4.6. Bunch weight

Bunch weight of individual treatment from the all plants was recorded at the time of crop harvest.

3.2.4.7. Number of hands

Number of hands in each bunches of the four plants were recorded and averaged.

3.2.4.8. Number of fingers

Number of fingers in each bunch was counted at the time of harvest of individual plants

3.2.4.9. Weight of fingers

Average weights of fingers of four plants in the plot were calculated by taking the weight of finger from the top, middle and bottom of each of the bunch and averaged

3.2.4.10. Length of fingers

Average length of fingers was calculated by measuring the length of the finger in the topmost bunch, middle of bunch and the lower end of the bunch and there averages were worked out and recorded.

3.2.4.11. Girth of fingers

Average breadth of fingers was calculated by measuring the breadth of the fingers in the topmost, middle and lower bunches and taking its mean.

3.2.4.12. Days to bunch emergence

Number of days to bunch emergence of individual treatments from the date of planting was recorded.

3.2.4.13. Days to harvest

Number of days taken from the date of planting to the date of harvest was recorded for individual treatments.

3.2.4.14. Incidence of pest and disease

Incidence of any pest and diseases were regularly monitored and recorded from hardening to crop harvest.

3.3. Quality parameters

Quality parameters of banana such as TSS, vitamin C content, titrable acidity and reducing sugar content were analyzed using standard procedures. Pulp to peel ratio was also found out.

3.3.1. Total Soluble Solids (TSS)

Total soluble solids of the fully ripened banana fruits were analyzed using hand held portable refractometer and the results were expressed in terms of ^{o}brix.

3.3.2. Vitamin C

Vitamin C (ascorbic acid) content of the fully ripened banana fruits were analyzed by taking 10 mL of the aliquot from 50 mL of solution containing 5 mL of fruit pulp and oxalic acid which was titrated against the 2,4-Dichlorophenol indophenol dye solution until a pink color was obtained which persists for 15 seconds. The results were expressed in terms of mg of vitamin C/100g of fruit.

3.3.3. Acidity

Titrable acidity of the fully ripened banana fruits were analyzed by grinding 50 g of fruit and 5 mL of the pulp was transferred to a 50 mL standard flask and volume was made up and from that 10mL of the extract was taken and titrated with 0.1 N NaOH using phenolphthalein indicator till the appearance of light pink colour which is the end point, and the titrable acidity was worked out and the results were expressed as percentage of malic acid.

3.3.4. Reducing sugar content

Reducing sugar content were determined by titrating fruit juice with Fehling solution A and B as per procedure given by Ranganna (1986) and the results were expressed in percentage.

3.3.5. Pulp to peel ratio

Weight of the pulp and peel were recorded separately and relative proportion of pulp and peel were recorded.

3.4. Soil sample collection

Soil samples for laboratory analysis were collected before the start of the field experiment and from each treatment after the crop harvest. They were analyzed for various chemical properties as per the standard procedures as given in table 1. Properties of the initial soil sample are given in table 2.

3.5. Plant sample collection

Index leaf (third leaf from the top at the time of bunch emergence) for plant sample analysis were collected at the time of harvest and analyzed the status of macro and micro nutrient in it using standard procedures as given in table 3.

3.6. Statistical analysis

The data obtained from polybag and field were analyzed statistically and tested for its significance using WASP 2.0 software given by ICARGOA.

Table 1: Analytical methods followed for soil analysis

Sl. No.	Parameter	Method	Reference
1	pH	pH meter	Jackson (1958)
2	EC	Conductivity meter	Jackson (1958)
3	Organic carbon	Chromic acid wet digestion method	Walkley and Black (1934)
4	Available N	Alkaline permanganate method	Subbaih and Asija (1956)
5	Available P	Bray extraction and photoelectric colorimetry	Jackson (1958)
6	Available K	Flame photometry	Pratt (1965)
7	Available Ca	Atomic absorption Spectroscopy	Jackson (1958)
8	Available Mg	Atomic absorption Specroscopy	Jackson (1958)
9	Available S	Photoelectric colorimetry	Massouni and Cornfield (1963)
10	Available Fe	Atomic absorption Specroscopy	Sims and Johnson (1991)
11	Available Mn	Atomic absorption Specroscopy	Sims and Johnson (1991)
12	Available Zn	Atomic absorption Spectroscopy	Emmel *et al.* (1977)
13	Available Cu	Atomic absorption Spectroscopy	Emmel *et al.* (1977)
14	Available B	Photoelectric colorimetry	Binghum (1982)

Table 2: Properties of the initial soil sample

Sl. No.	Parameter	Value
1.	pH	5.51
2.	EC (dS/m)	0.155
3.	Organic carbon (%)	0.193
4.	Available N (kg/ha)	235
5.	Available P_2O_5(kg/ha)	154
6.	Available K_2O (kg/ha)	141.26
7.	Available Ca (mg/kg)	43.75
8.	Available Mg (mg/kg)	11.26
9.	Available S (mg/kg)	5.00
10.	Available Fe (mg/kg)	10.66
11.	Available Mn (mg/kg)	28.26
12.	Available Zn (mg/kg)	3.83
13.	Available Cu (mg/kg)	6.8
14.	Available B (mg/kg)	0.16

Table 3: Analytical methods followed in leaf analysis

Sl. No.	Parameter	Method	Reference
1	Total N	Modified Kjeldhal digestion method	Jackson (1958)
2	Total P	Vanadomolybdate yellow colour method	Piper (1966)
3	Total K	Flame photometry	Jackson (1958)
4	Total Ca	Atomic absorption Spectroscopy	Issac and Kerber (1971)
5	Total Mg	Atomic absorption Specroscopy	Issac and Kerber (1971)
6	Total S	Turbidimetric method	Bhargava and Raghupathy (1995)
7	Total Fe	Atomic absorption Specroscopy	Piper (1966)
8	Total Mn	Atomic absorption Specroscopy	Piper (1966)
9	Total Zn	Atomic absorption Spectroscopy	Emmel *et al.* (1977)
10	Total Cu	Atomic absorption Spectroscopy	Emmel *et al.* (1977)
11	Total B	Azomethane - H colorimetric method	Binghum (1982)

Plate 1a: Fortified potting mixture

Plate 1b: Primary hardening

Plate 1c: Secondary hardening of TC plants

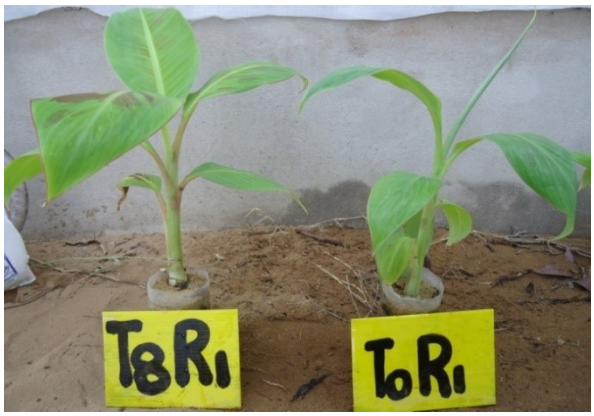

Plate 1d: Treatment vs control

Plate 1. Hardening of tissue culture banana var. Nendran

Plate 2a: Field view - two months after planting

Plate 2b. Field view after bunch emergence

Plate 2. Field view of the experimental plot at RARS farm, Nileshwar

Results

4. RESULTS

An investigation was carried out to examine the effect of calcium, magnesium, sulphur and boron application in tissue culture banana (var. Nendran) during hardening stage and in field condition. The results of biometric observations (taken after hardening and in field conditions) and various soil, leaf and fruit characteristics were statistically analysed and the results are presented below.

4.1 HARNENING STUDIES

The hardening studies of tissue culture raised banana plants were conducted in the poly house of College of Agriculture, Padannakkad, Kerala with fortified potting mixture containing various treatment combinations of calcium, magnesium, sulphur along with boron foliar spays. The experiment was intended to evaluate the role of these nutrients on performance of plants in hardening stage. Periodic biometric observations were made. Detailed observations and chemical analysis were done in the field experiment. The results of biometric observations of hardening studies taken at the end of two months are given below.

4.1.1. Leaf number

The results of the effect of potting mixture fortification with secondary nutrients and boron are given in table 4. All the treatments were found to be superior to the control. Among the treatments, T_{10} (150 ppm Ca, 25 ppm Mg, 25 ppm S and 0.5 % B) recorded maximum number of leaves (7.07) which was on par with treatments T_8, T_9 and T_{11}. Control recorded minimum leaf number (5.07).

4.1.2. Leaf length

There were significant differences among the treatments. Treatments T_{10} (19.63 cm) and T_8 (19.54 cm) recorded maximum leaf length which was on par with control (19.38 cm) and T_1 recorded minimum leaf length.

4.1.3. Leaf breadth

Maximum leaf breadth was recorded in T_{10} (6.73 cm) which was on par with T_8 (7.47 cm) and T_7 (7.13 cm) and T_1 recorded minimum leaf breadth (5.67 cm). Control was having 6.67 cm.

4.1.4. Plant height

Plant height showed significant difference among the treatments. T_7, T_8 and T_{10} exhibited higher plant height which was on par with that of control. Other treatments showed lower plant height.

Table 4: Effect of potting mixture fortification with secondary nutrients and boron sprays

Treatments	Leaf number	Leaf length (cm)	Length breadth (cm)	Plant height (cm)
T_1	6.467	14.113	5.673	9.400
T_2	6.267	16.833	6.700	11.933
T_3	6.400	15.567	6.213	11.233
T_4	6.467	16.340	6.280	11.667
T_5	6.533	15.873	6.080	11.300
T_6	6.067	15.220	5.800	9.700
T_7	6.533	17.533	7.127	13.493
T_8	6.867	9.540	7.467	13.433
T_9	6.600	16.207	6.420	10.367
T_{10}	7.067	19.633	7.633	12.800
T_{11}	6.867	16.020	6.233	9.533
T_{12}	6.400	16.938	6.393	10.533
T_{13}	6.333	15.813	6.087	10.900
T_{14}	6.067	15.387	5.893	10.100
T_{15}	6.333	15.200	5.867	9.200
T_{16}	6.283	15.518	5.858	10.683
T_{17}	5.067	19.387	6.667	13.733
CD (5%)	0.473	1.794	0.838	1.682

4.2. FIELD EXPERIMENT

The field experiment was conducted at Regional Agricultural Research Station farm at Nileshwar to study the effect of soil application of calcium, magnesium, sulphur and boron foliar sprays in tissue culture banana. Results of various biometric observations as well as detailed soil, leaf and fruit analysis were done. The data were subjected to statistical analysis and the results are presented below.

4.2.1. Effect on vegetative characters

4.2.1.1. Plant height

Observations on plant height, pseudostem girth at 30cm and leaf number taken at the time of shooting and number of suckers produced at the time of harvest were recorded. The data after statistical analysis is presented in table 5. Plant height at the time of shooting was taken from the ground upto the peduncle. There was no significant difference between the treatments. T_{12} recorded maximum plant height of 2.95 m while T_{13} recorded minimum plant height of 2.71 m whereas for control it was 2.80 m.

4.2.1.2. Pseudostem girth

Psuedostem girth at 30cm height at the time of shooting was found to be highest in T_3 (59.65 cm) followed by T_9 (59.24 cm) and lowest girth was in T_6 (57.12 cm). However, there was no significant difference between the treatments and control.

4.2.1.3. Leaf number

Leaf number exhibited significant differences among the treatments at 5% and 1% level of significance. T_{12} recorded highest leaf number (15.125) which was on par with T_{10} (14.96), T_5 (14.92), T_4 (14.75), T_{13} (14.75), T_6 (14.71), T_{16} (14.57) and T_7 (13.75) recorded minimum leaf number.

4.2.1.4. Number of suckers at harvest

Significant differences in average number of suckers produced at the time of harvest were found. Maximum number of suckers was in T_3 (7.50) and control recorded minimum of 5.75 suckers.

Table 5: Effect of secondary nutrients and boron application on growth parameters of banana

Treatments	Plant height (cm)	Pseudostem girth (cm)	Number of suckers	Number of leaves
T_1	2.839	57.575	7.250	14.250
T_2	2.776	58.192	7.000	13.833
T_3	2.778	59.650	7.500	14.083
T_4	2.862	58.767	6.333	14.750
T_5	2.839	57.342	5.917	14.917
T_6	2.801	55.117	6.417	14.708
T_7	2.699	55.492	6.667	13.750
T_8	2.861	57.625	7.000	14.333
T_9	2.818	59.242	6.390	13.917
T_{10}	2.810	58.658	6.750	14.958
T_{11}	2.812	57.383	6.417	14.250
T_{12}	2.950	57.492	6.500	15.125
T_{13}	2.704	57.392	6.333	14.750
T_{14}	2.686	57.933	6.417	14.417
T_{15}	2.853	58.033	6.833	14.417
T_{16}	2.819	57.758	6.750	14.569
T_{17}	2.804	58.225	5.750	14.000
CD (5%)	NS	NS	0.074	0.659
SE	0.121	2.166	-	-

4.2.2. Effect on yield attributes

4.2.2.1. Weight of male bud

Weight of male bud at the time of detachment was noted and compared. There was no significant difference between the average weight of male bud. Highest weight was obtained in T_6 (2.154 kg) followed by T_{11} (1.889 kg) and minimum was found in control (1.471 kg), T_4 (1.311 kg), T_7 (1.256 kg), and T_9 (1.272 kg).

4.2.2.2. Bunch weight

Bunch weight of plant is the major economic factor considered in banana cultivation. There was a significant influence of treatments on bunch weight and the results are presented in table 6. All treatments with secondary nutrients and boron were found to be superior to the control which had an average yield of 10.34 kg. Highest bunch weight was recorded in T_4 (75 g Ca + 25 g Mg + 50 g S + 0.5 % B) with 12.97 kg which was on par with treatments T_3 (12.66 kg), T_1 (12.44 kg), T_5 (12.26 kg), T_{12} (11.98 kg), T_2, T_{15}, T_8 and T_{11}. Plate 3 and 4 shows comparison between bunch weight of selected treatments and control.

4.2.2.3. Number of hands

Number of hands per bunch and fingers per bunch are yield attributing factors. The observations made on these parameters are compared and the results are presented in table 6 and 7. Average number of hands showed significant differences among treatments. The highest number of hands was noticed in T_{11} (5.583) and all other treatments except T_7, T_3, T_{16}, T_{13}, T_6, T_{10} and T_2.While that of control it was (5.167). Lowest number was in T_2 (4.50). From the plate 3b it is clear that in the control plants the first two hands were devoid of fingers when compared to plants receiving treatments of secondary nutrients and boron.

Table 6: Effect of secondary nutrients and boron application on yield parameters of banana

Treatments	Bunch weight (kg)	Malebud weight (kg)	No. of hands
T_1	12.443	1.533	5.500
T_2	11.893	1.575	4.500
T_3	12.663	1.799	5.000
T_4	12.917	1.311	5.250
T_5	12.260	1.839	5.111
T_6	11.187	2.154	4.611
T_7	10.830	1.256	5.000
T_8	11.653	1.342	5.167
T_9	11.010	1.272	5.167
T_{10}	10.900	1.875	4.556
T_{11}	11.620	1.889	5.583
T_{12}	11.980	1.654	5.083
T_{13}	10.833	1.814	4.889
T_{14}	11.417	1.650	5.250
T_{15}	11.753	1.546	5.167
T_{16}	11.503	1.704	4.917
T_{17}	10.340	1.471	5.167
CD (5%)	1.292	NS	0.556
SE	-	0.342	-

4.2.2.4. Number of fingers

Total number of fingers per bunch was recorded and compared. The effects of treatment application on the number of fingers are presented in table 7. Treatment effects were significant in case of average number of fingers in bunch. Maximum number of fingers was found in T_4 which recorded 52.67 whereas T_6 showed minimum with 44.33 fingers. The control recorded 48.33 number of fingers.

4.2.2.4. Average weight of fingers

The average finger weight was calculated by taking the best finger in each of the hands per bunch and averaged. The effect of treatments on average weight of fingers was also significant. T_{10} produced maximum average finger weight of 281.33 g whereas minimum was in T_{13} with 214.33 g. For control it was 242.67 g. Treatment effects on average weight of fingers are presented in table 7.

4.2.2.5. Average length of fingers

Average length of fingers also exhibited no significant differences among treatments as well as control. In T_3 maximum finger length of 23.19 cm and in T_{16} minimum length of 20.88 cm was observed. For control it was 21.4 cm. Plate 5 shows finger characteristics of selected treatment and control.

4.2.2.6. Average girth of fingers

Treatment effects on finger girth at middle portion was also non significant. Maximum finger girth was noticed in T_5 (14.98 cm) whereas minimum finger breadth was seen in T_7 (14.10 cm). Control showed 14.6 cm. Plate 5 shows finger characteristics of selected treatment and control. The effect of treatment application on the length and girth of fingers are presented in table 7.

Table 7: Effect of secondary nutrients and boron application on finger characteristics of banana

Treatments	Finger number	Average weight (g)	Average length (cm)	Average breadth (cm)
T_1	52.000	230.000	22.217	14.467
T_2	46.000	269.667	22.510	14.887
T_3	48.667	276.000	23.193	14.817
T_4	52.667	241.667	22.610	14.580
T_5	49.000	236.333	22.747	14.983
T_6	44.333	239.333	22.417	14.773
T_7	47.333	236.000	21.583	14.097
T_8	48.333	232.667	22.593	14.717
T_9	50.333	222.000	21.690	14.110
T_{10}	42.000	281.333	22.540	14.790
T_{11}	52.000	239.000	22.653	14.853
T_{12}	50.333	215.667	22.053	14.203
T_{13}	49.000	214.333	21.387	14.343
T_{14}	47.667	228.667	22.450	14.387
T_{15}	48.667	255.667	22.780	14.597
T_{16}	45.667	243.000	20.883	14.200
T_{17}	48.333	242.667	21.400	14.610
CD (5%)	0.465	3.222	NS	NS
SE	-	-	0.840	0.400

4.2.2.7. Days to bunch emergence

Effect of treatment application on the number of days taken from planting to bunch emergence are presented in table 8. Treatments were found to be non significant in influencing the days to bunch emergence. T_7 produced earliest bunch in 170.67 days while T_5 in 185.33 days which was the late one. The control took 175.56 days. So bunching happened in 170-185 days and the treatments seem to have no effect.

4.2.2.8. Days to bunch harvest

Treatment effects on influencing the days to bunch harvest was also non significant. T_6 took maximum days from planting to harvest (282.89) while T_9 took minimum of 260.44 days. Effect of treatment applications on the number of days taken from planting to harvest are presented in table 8.

4.2.2.9. Incidence of pest and disease

The observations were also made on the incidence of pest and diseases. The major problem noticed was sigatoka leaf spot caused by *Mycosphaerella musicola*. Control measures were taken uniformly for that, after recording the number of affected leaves per plant. The mean of the infested leaves per plant were taken and where analysed statistically. Eventhough statistically non significant, control plants showed greater degree of infestation compared to treatments. Effect of treatment applications on the number of sigatoka infested leaves are presented in table 8.

Table 8: Effect of secondary nutrients and boron application on days to bunch emergence, days to harvest and pest and disease incidence of banana

Treatment	Days to bunch emergence	Days to harvest	Pest and disease incidence
T_1	176.833	270.833	2.625
T_2	173.667	265.333	2.125
T_3	177.833	272.917	2.021
T_4	177.889	271.417	2.292
T_5	184.333	281.889	1.979
T_6	181.667	282.889	1.667
T_7	170.667	264.556	2.667
T_8	173.000	263.167	2.354
T_9	176.333	260.444	2.229
T_{10}	180.667	282.889	1.917
T_{11}	178.500	272.667	3.021
T_{12}	176.667	271.583	2.458
T_{13}	174.667	282.111	1.500
T_{14}	171.167	272.750	1.833
T_{15}	176.333	272.972	2.458
T_{16}	174.833	266.333	2.333
T_{17}	175.556	261.667	3.208
CD (5%)	NS	NS	NS
SE	4.004	8.819	0.616

Plate 3 a. Best Treatment T_4

Plate 3 b. Control

Plate 3 c. Second best treatment

Plate 3 d. Control

Plate 3: Best treatment bunches vs control

Plate 4 a. Control

Plate 4 b. Treatment T_{12}

Plate 4 c. Control

Plate 4 d. Treatment T_{14}

Plate 4. Good treatment bunches vs control

Plate 5a.Finger characteristics of best treatment vs control

5b. Finger of the best treatment

5c. Treatment T_6

Plate 5. Finger characteristics of treatment and control

4.3. FRUIT CHARACTERISTICS

The effects of application of calcium, magnesium, sulphur and boron on fruit characteristics of banana were examined. Various quality parameters like titrable acidity, vitamin C, total soluble solids, reducing sugar content and pulp to peel ratio were analysed and the results are presented below.

4.3.1. Titrable acidity

The influence of treatment application on titrable acidity percentage of ripened banana fruits are presented in table 9. The effect of treatments was found to be statistically significant. Lesser acidity indicates better quality and average titrable acidity recorded in the treatment applied fruits were 0.68 % as against 0.81 % noticed in control. Maximum acidity was reported in T_4 (0.86 %) followed by T_3 (0.83 %) and control (0.81 %) while T_2 recorded minimum acidity (0.53 %).

4.3.2. Vitamin C

Vitamin C content of the fruits were measured in the pulp and compared. There were significant differences among the treatments when compared to control. Among the treatments, highest vitamin C content was in T_5 (15.39 mg/100g), and lowest content in T_7 (8.03 mg/100g). For control 9.03 mg/100g was observed. Treatments which were given first level of Ca showed a vitamin C content of 10.28 mg/100g and second level 10.44 mg/100g. In case of Mg it was 10.20 mg/100g and 10.57 mg/100g in the first and second levels respectively. The effect of treatment application on the vitamin C content of the ripened banana fruit are presented in table 9.

4.3.3. TSS

Total soluble solid content of fruits were not significantly influenced by the treatment application. However, maximum TSS content was found in T_6 (30.17 ^{0}brix) and minimum in T_{11} (27.83 ^{0}brix) while for control it was 28.67 ^{0}brix. The results are presented in table 9.

Table 9: Effect of secondary nutrients and boron application on titrable acidity, vitamin C content, and TSS of ripened banana fruit

Treatments	Acidity (%)	Vitamin C (mg/100g)	TSS (^{0}brix)
T_1	0.710	10.034	28.000
T_2	0.350	8.696	29.667
T_3	0.828	10.034	28.500
T_4	0.858	9.365	29.833
T_5	0.562	15.386	28.833
T_6	0.710	12.041	30.167
T_7	0.527	8.027	28.000
T_8	0.464	8.696	28.333
T_9	0.695	10.703	28.000
T_{10}	0.695	10.703	29.667
T_{11}	0.680	10.034	27.833
T_{12}	0.784	12.041	29.667
T_{13}	0.621	10.034	29.667
T_{14}	0.651	10.703	30.000
T_{15}	0.710	10.034	28.267
T_{16}	0.784	10.703	30.000
T_{17}	0.813	9.031	28.667
CD (5%)	0.045	0.562	NS
SE	-	-	1.299

4.3.4. Reducing sugar content

The effect of treatment application was highly evident in case of reducing sugar content of fruits and the treatments showed superior and significant differences. Among the treatments, T_6 exhibited highest reducing sugar content of 8.33 percent whereas control reported lowest reducing sugar content of 4.39 percent. The results of treatment application on the reducing sugar content of the ripened banana fruits are presented in table 10.

4.3.5. Pulp peel ratio

The effect of treatment application was evident in case of pulp peel ratio also. Treatments showed significant differences and were superior to control. Among the treatments, lowest pulp peel ratio was in control (3.38) and highest ratio was observed in T_{16} (6.77) and T_6 (6.38). Table 10 shows the effect of treatments on the reducing sugar content and pulp to peel ratio of the ripened banana fruits.

Table 10: Effect of secondary nutrients and boron application on reducing sugar content and pulp to peel ratio of ripened banana fruit

Treatment	Reducing sugar (%)	Pulp to peel ratio
T_1	8.133	4.124
T_2	6.250	5.419
T_3	7.576	4.069
T_4	5.319	3.776
T_5	6.757	3.611
T_6	8.333	6.384
T_7	5.319	3.485
T_8	5.556	4.139
T_9	6.250	4.504
T_{10}	6.250	4.203
T_{11}	4.545	5.538
T_{12}	4.464	4.473
T_{13}	5.556	4.608
T_{14}	5.435	5.427
T_{15}	6.250	5.812
T_{16}	7.813	6.773
T_{17}	4.386	3.381
CD (5%)	0.416	0.340

4.4. SOIL CHARACTERISTICS

4.4.1. pH

The results of soil pH, EC and OC examined for plots which received various treatment combinations are presented in table 9. The effect of treatment application on soil pH was statistically non significant. T_{13} had the lowest pH value of 4.64 and the highest was in T_{16} (5.06).

4.4.2. EC

Treatment application has significant effect on the electrical conductivity of soil. Average EC in the treatment plots were 0.208 dS/m compared to 0.124 dS/m in control plot. Highest EC was found in T_4 (0.365 dS/m) while lowest was in T_{16} (0.879 dS/m).

4.4.3. Organic carbon content

Organic carbon content of the soils was not influenced significantly by the treatment application. OC content varied from 0.28 % in T_7 and T_{14} to 1.22 % in T_5. On an average, OC content of treatments were 0.46 % and for control 0.29 %.

4.4.4. Available N

Table 10 shows the values of available N, P and K status (kg/ha) of the various plots. The effect of secondary nutrients and boron was found to be non significant in case of available N. Highest N content was found in T_{16} and lowest was in T_{12}.

4.4.5. Available P

The effect of treatment application was significant in case of available P content of soil and it varied from 203.36 kg/ha in T_{14} to 27.3 kg/ha in T_{16}. The average P content in treatment plots were 112.35 kg/ha compared to 172.48 kg/ha in control. Highest P content was seen in T_{14} which was on par with T_{15}, T_{17}, T_7, T_5, T_8, T_1 and T_6.

4.4.6. Available K

Available K levels in soil ranged from 205.17 kg/ha (T_3) to 394.64 kg/ha (T_6). Average K content of control was found as 215.26 kg/ha compared to 320.92 kg/ha in treatment plot. But the differences were found to be statistically non significant.

Table 11: Effect of secondary nutrients and boron application on soil pH, EC and OC

Treatments	pH	EC (dS/m)	OC (%)
T_1	4.700	0.215	0.435
T_2	4.803	0.198	0.597
T_3	4.907	0.177	0.215
T_4	4.823	0.365	0.172
T_5	4.757	0.247	1.222
T_6	4.710	0.169	0.495
T_7	4.890	0.193	0.280
T_8	4.763	0.121	0.462
T_9	4.707	0.194	0.726
T_{10}	4.770	0.269	0.269
T_{11}	4.860	0.192	0.731
T_{12}	4.693	0.279	0.215
T_{13}	4.637	0.199	0.403
T_{14}	4.673	0.215	0.280
T_{15}	4.883	0.203	0.403
T_{16}	5.063	0.879	0.587
T_{17}	4.697	0.124	0.290
CD (5%)	NS	0.131	NS
SE	0.132	-	0.356

Table 12: Effect of secondary nutrients and boron application on available N, P, K content of soil

Treatment	Available N(kg/ha)	Available P_2O_5 (kg/ha)	Available K_2O (kg/ha)
T_1	210.00	130.11	257.86
T_2	208.67	107.52	329.61
T_3	213.67	106.77	205.17
T_4	206.33	106.77	291.49
T_5	207.67	151.47	344.18
T_6	208.00	126.84	394.64
T_7	211.33	170.80	294.86
T_8	207.33	138.88	267.95
T_9	205.33	44.80	366.67
T_{10}	209.67	108.08	359.88
T_{11}	206.67	53.76	386.79
T_{12}	200.00	86.99	332.97
T_{13}	209.67	55.72	346.43
T_{14}	213.00	203.36	330.73
T_{15}	211.00	172.67	348.67
T_{16}	216.00	27.31	276.92
T_{17}	214.33	172.48	215.26
CD (5%)	NS	87.60	NS
SE	9.99	-	74.80

4.4.7. Available Ca

The effect of treatment application on the available Ca status of soil is presented in table 13. Available Ca content in the soil were statistically non significant, even then the available Ca content in treatment plots was higher with 47.89 mg/kg as against control with 41.85 mg/kg. Average Ca content ranged from 41.85 mg/kg in control to 57.83 mg/kg in T_7. Treatments which received first level of Ca (75 g/plant) showed an average content of 49.59 mg/kg and those with second level of Ca had 46.18 mg/kg of available Ca.

4.4.8. Available Mg

The differences among available Mg content of the soils which received treatment application to that of control was found to be statistically non significant. At the same time, the average Mg content ranged from 8.09 mg/kg in control plot to 9.62 mg/kg in treatment plots. Highest Mg content was found in T_7 (10.98 mg/kg) while lowest in control (8.09 mg/kg). Treatments which received first level of Mg (25 g/plant) showed an average Mg content of 9.44 mg/kg whereas for those with second level (50 g/plant) the average content was 9.81 mg/kg. The effect of treatment application on the available Mg status of soil is presented in table 13.

4.4.9. Available S

The influence of treatment applications on the available S status of soil are presented in table 13. Available S content in the soil was also found to be non significant. However, S content varied from 13.75 mg/kg in T_{17} to 26.67 in T_{16}. Average S content in plots which received first and second level of S treatment was 21.58 mg/kg and 22.43 mg/kg.

Table 13: Effect of secondary nutrients and boron application on available Ca, Mg, and S content in soil (mg/kg)

Treatment	Available Ca	AvailableMg	Available S
T_1	48.567	9.089	25.000
T_2	51.100	9.511	21.250
T_3	49.600	9.178	16.000
T_4	46.750	9.844	16.300
T_5	45.283	9.133	24.167
T_6	51.333	8.978	20.667
T_7	57.833	10.978	23.667
T_8	46.300	10.422	24.000
T_9	51.383	9.689	17.667
T_{10}	46.883	9.400	23.250
T_{11}	42.267	9.733	24.333
T_{12}	41.283	9.111	25.000
T_{13}	42.750	8.956	25.667
T_{14}	48.633	10.733	15.000
T_{15}	46.600	8.956	23.500
T_{16}	49.633	10.356	26.667
T_{17}	41.850	8.093	13.750
CD (5%)	NS	NS	NS
SE	5.765	0.859	10.57

4.4.10. Available Cu

The results of soil analysis for available status of Cu, Fe, Zn, Mn and B are presented in table 12. The treatment T_{13} (7.24 mg/kg) recorded highest available Cu content in soil compared to all other treatments. However, it was on par with all other remaining treatments except T_6, T_8, T_7, T_{12}, T_2, T_{15}. The average Cu content in treatment applied plots were 6.29 mg/kg as against 6.68 mg/kg in control.

4.4.11. Available Fe

The effect of treatment application on available Fe content of soil was non significant. Fe content ranged from 9.39 mg/kg in T_{16} to 30.71 mg/kg in T_7. Average Fe content of treatment plots were 12.95 mg/kg while for that of control it was 12.18 mg/kg with no significant difference.

4.4.12. Available Mn

Available Mn content in soil was not significantly influenced by the treatments. It ranged from 12.58 mg/kg in T_{16} to 29.88 mg/kg in T_4. The average Mn content of treatment plots were 23.04 mg/kg as against 12.49 mg/kg in control.

4.4.13. Available Zn

Significant differences were observed in available Zn content of soil as a result of treatment application. The differences were so prominent and were significant at 1% as well as 5% level of significance. Highest Zn content was recorded in T_8 (5.13 mg/kg) which was on par with treatments T_2, T_6, T_4, T_{15}, T_3, T_{10} and T_{11}. The average Zn content in treatment plots was 3.58 mg/kg while that of control plot it was 3.10 mg/kg.

4.4.14. Available B

Available B content in soil was not statistically significant. Average content ranges from 0.13 mg/kg in control plot to 0.58 mg/kg in T_{16}. Average B content in treated plots was 0.40 mg/kg.

Table 14: Effect of secondary nutrients and boron application on available micronutrient content of soil (mg/kg)

Treatments	Cu	Fe	Mn	Zn	B
T_1	6.720	13.422	16.511	3.120	0.395
T_2	5.287	25.822	24.333	4.724	0.478
T_3	6.409	21.044	24.178	4.049	0.354
T_4	6.313	13.822	29.844	4.231	0.277
T_5	7.156	25.267	24.978	2.556	0.404
T_6	6.089	24.178	30.133	4.376	0.355
T_7	5.673	30.711	25.444	3.569	0.300
T_8	5.884	17.311	23.978	5.131	0.517
T_9	7.109	13.200	19.444	2.822	0.363
T_{10}	6.371	16.556	26.222	4.009	0.394
T_{11}	6.871	23.244	23.444	3.796	0.273
T_{12}	5.500	26.489	23.956	2.549	0.450
T_{13}	7.244	21.600	23.133	3.367	0.388
T_{14}	6.313	19.178	19.267	2.567	0.445
T_{15}	5.231	18.067	21.133	4.160	0.406
T_{16}	6.511	9.398	12.578	2.251	0.577
T_{17}	6.689	12.178	14.489	3.104	0.130
CD (5%)	1.143	NS	NS	1.477	NS
SE	-	6.298	5.745	-	0.106

4.5. LEAF CHARACTERISTICS

The chemical analysis of banana leaf samples was conducted in order to examine the plant nutrient status. Leaf samples were collected at the time of harvest, dried in oven and were powdered and analysed using standard analytical procedures explained in the materials and methods. The results obtained after statistical analysis are presented in tables 14, 15 and 16 below.

4.5.1. Nitrogen

Table 15 shows the effect of secondary nutrients and boron application on the N status of banana leaf at the time of harvest. There was no significant difference in the N content of the leaves among the treatments and control. T_5 recorded highest N content whereas T_{16} recorded lowest N content in the leaves.

4.5.2. Phosphorous

The effect of treatment application on the banana leaf P content is presented in table 15. Phosphorous content of the leaf was not significantly influenced by the application of secondary nutrients and boron. P content ranged between 0.11 % in control to 0.15 % in T_{14}. On an average P content in leaves of treatment applied plants was found to be 0.12 %.

4.5.3. Potassium

There was no statistical difference on effect of treatment application influencing the leaf K content. Potassium content of the leaves ranged from 1.92 % in T_3 to 2.42 % in T_6. As a whole K content in treated plants were 2.12 % as against 2.28 % in control with no significant difference. The effect of secondary nutrients and boron application on the K content of banana leaf at the time of harvest is presented in table 15.

Table 15: Effect of secondary nutrients and boron application on N, P, K content of banana leaf

Treatments	N (%)	P (%)	K (%)
T_1	2.543	0.128	2.383
T_2	2.514	0.113	2.017
T_3	2.563	0.118	1.917
T_4	2.839	0.123	2.250
T_5	2.921	0.113	2.233
T_6	2.673	0.126	2.417
T_7	2.871	0.130	1.483
T_8	2.781	0.117	2.150
T_9	2.652	0.136	2.217
T_{10}	2.527	0.126	1.983
T_{11}	2.771	0.115	2.117
T_{12}	2.613	0.126	2.133
T_{13}	2.688	0.108	2.367
T_{14}	2.591	0.147	2.283
T_{15}	2.755	0.128	1.967
T_{16}	2.365	0.116	2.150
T_{17}	2.504	0.105	2.283
CD (5%)	NS	NS	NS
SE	0.227	0.021	0.313

4.5.4. Calcium

The effect of secondary nutrients and boron application on the leaf Ca content of banana is presented in table 16. The effect of calcium content in leaves as influenced by different treatments showed statistically significant differences. However, it ranged from 3.10 % in T_{17} to 4.95 % in T_2. On an average, Ca content in the treatment applied leaves were 4.20 % as against 3.10 % in control. Treatments which received first level of Ca (75 g/plant) showed an average Ca content of 4.13 % while that for second level (150 g/plant) it was 4.28 %.

4.5.5. Magnesium

The effect of secondary nutrients and boron application on the leaf Mg content of banana is presented in table 16. There was significant difference among magnesium content of the leaves as influenced by the treatments. It ranged from 341 ppm in case of control to 488 ppm in case of T_7. Average Mg content of treatment applied plots were 458.8 ppm whereas for control plot the content was 371 ppm. Leaf Mg content of plants supplied with first level of Mg (25 g/plant) was found to be 463 ppm and that for second level Mg (50 g/plant) it was 451ppm.

4.5.6. Sulphur

The effect of secondary nutrients and boron application on the leaf S content of banana is presented in table 16. The differences among treatments with respect to sulphur content of the leaves were also significant. S content ranged from 0.14 % in T_9 to 2.91 % in T_{14}. Average S content in leaves which had treatment was found to be 0.89 % while that for control it was only half registering 0. 45 %. Average S content in leaves which received first level of S (25 g/plant) was 0.85 % and second level of S (50 g/plant) was 0.92 %.

Table 16: Effect of secondary nutrients and boron application on Ca, Mg, S content of banana leaf

Treatments	Ca (%)	Mg (ppm)	S (%)
T_1	4.807	482.667	0.197
T_2	4.953	482.000	0.189
T_3	3.853	456.667	1.181
T_4	4.287	438.667	0.683
T_5	3.400	441.333	0.704
T_6	4.427	479.333	1.256
T_7	3.507	488.000	1.832
T_8	3.840	462.667	0.227
T_9	4.380	464.667	0.136
T_{10}	4.053	448.000	1.213
T_{11}	4.233	461.333	0.213
T_{12}	4.933	476.000	2.805
T_{13}	3.847	443.333	0.200
T_{14}	4.253	432.667	2.907
T_{15}	4.887	438.000	0.200
T_{16}	3.680	446.000	0.219
T_{17}	3.100	341.000	0.452
CD (5%)	0.927	58.491	1.753

4.5.6. Copper

The effect of secondary nutrients and boron application on the micronutrient content of banana leaf at the time of harvest is presented in table 17. Copper content of the leaves was not significantly influenced by the treatment application. Cu content ranged between 76.1 ppm in T_{13} to 120.7 ppm in control. Average Cu content in treatment applied one was 96.8 ppm and for control 120.7 ppm.

4.5.7. Iron

Significant differences were noticed among the leaf Fe contents. Highest Fe content was found in T_2 (1787 ppm) which was on par with treatments T_9, T_5, T_{13}, T_1, T_8, T_6, T_{11} and T_3 and lowest Fe content was in control (874 ppm).

4.5.8. Zinc

There was no significant difference between the Zn content of the leaves. However it was in the range of 25.8 ppm (T_{11}) to 45.3 ppm (T_{10}). Zinc content of treatment applied one was 25.9 ppm as against 20.2 ppm in control.

4.5.9. Manganese

Manganese content of the leaves was statistically non significant. It was in the range of 2816 ppm (T_{14}) to 7020 ppm (T_{17}). The average Mn content of treatments were 4185 ppm and for control it was 7020 ppm.

4.5.10. Boron

Leaf boron content showed significant differences among the treatments. Highest B content was found in T_{16} (51.3 ppm) and the lowest B content was found in control (T_{17}) with 16.3 ppm. Increasing the levels of boron from first to second increased the leaf B status from 32.4 ppm to 43.9 ppm.

Table 17: Effect of secondary nutrients and boron application on micronutrient content of banana leaf (ppm)

Treatment	Cu	Fe	Zn	Mn	B
T_1	79.2	1551.3	11.9	4093.3	30.25
T_2	93.7	1786.7	47.4	5440.0	33.41
T_3	100. 3	1332.7	14.7	4220.0	29.54
T_4	105.0	1222.0	18.5	5453.3	39.40
T_5	85.8	1632.7	13.5	4600.0	30.46
T_6	89.3	1433.3	12.7	3166.7	44.31
T_7	108.7	1252.0	27.4	5595.0	29.43
T_8	96.1	1482.0	3.3	3853.3	42.14
T_9	79.1	1759.3	13.0	3334.7	28.46
T_{10}	104.1	1241.3	45.3	4498.7	43.41
T_{11}	119.5	1370.7	25.8	4720.0	31.36
T_{12}	110.6	936.0	25.2	4853.3	47.34
T_{13}	76.1	1591.3	6.2	3484.7	39.34
T_{14}	108.0	1166.0	44.5	2816.0	50.50
T_{15}	96.6	1251.3	5.9	3406.7	40.45
T_{16}	96.4	1210.0	23.4	3426.7	51.30
T_{17}	120.7	874.0	20.2	7020.0	16.31
CD (5%)	NS	459.9	NS	182.0	2.32
SE	17.3	-	48.9	-	-

Discussion

5. DISCUSSION

Discussion of the results of investigation carried out at College of Agriculture, Padannakkad and Regional Agricultural Research Station farm of Nileshwar to study the effect of soil application of calcium, magnesium, sulphur and boron foliar sprays on tissue culture banana cv. Nendran are presented below. The investigation comprised of hardening studies of tissue culture banana plants using fortified potting mixture containing secondary nutrients and boron application as foliar sprays. The hardened plants were then planted in the field and same nutrients (calcium, magnesium, sulphur as soil application and boron as foliar sprays) were applied to study its effect on plant growth, yield characteristics and fruit quality.

5.1. HARDENING STUDIES

Soil application of calcium, magnesium, sulphur and foliar application of boron significantly increased the plant growth characteristics like leaf number, leaf length, leaf breadth and plant height (Table 4). Increases recorded in these biometric observations might be due to the beneficial effect of secondary nutrients and boron since their widespread deficiencies and their low content had been reported (KSPB, 2013).

Calcium might have aided in promoting cell elongation and cell division which would have promoted better leaf growth. Karaivazoglou *et al.* (2007) in tobacco reported that Ca application as lime treatments had significantly increased plant height. The increase in plant height can also be attributed to increased cell division and cell elongation induced by the interaction between Ca and auxin (Hamzawi, 2010).

The application of magnesium would have increased the chlorophyll content and photosynthetic rate of the plants which would have increased the rate of leaf production. Turner and Barkus (1980) reported the reduction in leaf area and number of live leaves in Mg deficient banana leaves which clearly

indicates the beneficial effect of Mg in enhancing the leaf production, as noticed in the present study.

Mostafa and Kader (2006) also cited that the application of sulfur was more effective in enhancing plant growth parameters like pseudostem length, circumference, number of green leaves at bunch emergence and leaf chlorophyll content which might be due to enhanced nitrogen fertilizer use and better plant metabolism. These results are also corroborated with those obtained by Turner *et al*. (1989), Abdkader *et al.* (1990), Abouaziz *et al.* (1993), Pertiz and Das (1998), and Hosam (2002).

Boron being a regulator of cell elongation and/or cell division (Mouhtaridou *et al.,* 2004), might have stimulated the meristematic growth (Bohnsack and Albert, 1977). Besides, B cross-links two rhamnogalacturonan II monomers by a borate bridge and provides stability to the cell wall matrix. Thus the presence of B would have increased the rate of formation and stability of this cross-link (O'Neill *et al.,* 2004), which might have lead to better plant growth.

A notable observation during the investigation is that, compared to the control plants, which were tall and flaggy the plants receiving secondary nutrients and boron were found to be more vigourous and sturdy (Plate 1d). This might be due the beneficial effect of calcium on plant vigour and stalk stiffness. Similar results were also reported by Follet *et al.* (1981) in cereals. This can also be due to the role played by Ca during cell wall formation because the acidic pectin residues (e.g., galacturonic acid) which are secreted as methyl esters, will be de-esterified by pectin methylesterase, liberating carboxyl groups, which bind Ca. Under low Ca situations the cell wall becomes more pliable and easily ruptured, whereas high concentrations rigidify the wall and make it less plastic. Besides these, within the complex framework of carbohydrates and proteins of the cell wall, there could be interactions between Ca and molecules other than pectins that could also contribute to cell wall structure and extensibility (Hepler, 2005). This can also make the plants more vigourous and sturdy.

5.2. FIELD EXPERIMENT

Tissue culture plants after hardening were planted out in the field and the similar treatments which were received during hardening stage were continued with respect to levels of secondary nutrients and micronutrient combinations. It was found that soil application of calcium, magnesium, sulphur and foliar application of boron significantly enhanced the vegetative characters like number of leaves at the time of shooting and yield characteristics like bunch weight, number of hands, number of fingers, average weight of fingers, and the average number of suckers produced. Quality parameters like acidity and vitamin C content, reducing sugar content and pulp to peel ratio also exhibited significant difference among the treatments. The quality characteristics were better in plants receiving the secondary and micronutrients. This might be due to the beneficial effect of Ca, Mg, S and boron.

5.2.1. Effect on plant growth parameters

The effect of secondary nutrients and boron on plant height, pseudostem girth and number of suckers was found to be non significant. It shows that secondary nutrients and boron application couldn't make any substantial effect on the above growth parameters.

Studies conducted by Spencer (1963) also indicated that Ca had no significant effect on influencing trunk diameter of orange trees. Studies of Prema (1992) also pointed that Mg treatments failed to influence the vegetative characteristics in banana. Nomura *et al.* in 2011 concluded that there was no effect of B application on banana plant growth parameters (height and pseudostem diameter) at the blooming stage. This may be due to the effect of B in increasing the number of fruit yield which reflected in eliminating vegetative growth, or may be due to the competition between fruit growth and vegetative growth. Such an effect could be also interpreted on the basis that fruit is considered as a strong sink for assimilates and hence, increasing B concentrations might have led to higher fruit yield and reduced vegetative

growth (Shazly and Kotb, 2011). A similar result of decreasing shoot length as a result of B application was also observed by Wojcik (1999) on prune trees.

Eventhough the effect of secondary nutrients and boron on plant height, pseudostem girth and number of suckers was found to be non significant, average number of leaves remaining at the time of shooting in the treatments was found to be significantly higher than that of control. The synthesis and transport of plant assimilates to the developing banana fruit is greatly affected by the retention of green leaves after the flowering stage, especially when assimilate flow from other plant parts becomes limiting (Kumar and Kumar, 2007). So it could have an indirect effect on the fruit yield which can be attributed to the positive effects of secondary nutrients and boron.

Chapman *et al.* (1965) also reported that Ca is required for the development of foliages and under situations of deficiency less foliage was produced in sweet orange. Hence Ca might have aided in promoting cell elongation and cell division and consequently in higher number of leaf production.

Application of magnesium would have increased the chlorophyll content and photosynthetic rate of the plants which in turn can enhance the rate of leaf production. The results are in harmony with those obtained by Mostafa *et al.,* 2007; Abdkader *et al.,* 1990. Turner and Barker (1983) also reported that low Mg supply reduced the mean leaf area growth rate and the number of live leaves present on the plants, whereas it had no effect on the number of sucker produced.

Mostafa and Kader (2006) also cited that the application of sulfur was more effective in enhancing number of green leaves at bunch shooting which might be due to enhanced nitrogen fertilizer use in soil and positive effects on plant metabolism. These results are in line with those obtained by Turner *et al*.

(1989), Kader *et al.* (1990), Abouaziz *et al.* (1993), Pertiz and Das (1998), and Hosam (2002).

Eventhough statistically non significant, control plants showed greater degree of sigatoka leaf spot infestation compared to treatments. Lower incidence of sigatoka leaf spot might be due to the better plant nutritional status of the crop since a balanced nutrition can results in high rates of leaf emergence, leading to better tolerance and possible escape from the effects of *Mycosphaerella* leaf spots. Vincente (2012) stated that plants with calcium deficiency exhibit more severe BSD symptoms which may can be attributed to the increased infestation in the control plots than treatment plots.

5.2.2. Effect of yield characteristics

Yield in bananas is a function of bunch weight and number of plants per hectare. Hence, any nutrient management study should aim at producing maximum bunch weight, so that, the productivity could be enhanced reasonably (Kumar and Kumar, 2008).

In the present study, soil application of calcium, magnesium and sulphur along with boron foliar sprays in tissue cultured banana cv. Nendran have shown positive and significant influence in the yield characteristics of the crop. Treatment application has significantly increased the bunch weight, number of hands, number of fingers, average weight of fingers and the number of sucker produced which are of paramount importance from the economic point of view.

Bunch weight in treated plants was found to be superior to that of control. Figure 2 shows the effect of different treatments on bunch weight. It was also found that increasing the Ca and Mg levels from first to second slightly reduced the yield whereas for S there was an improvement in bunch weight, the influence of B in first and second levels were more or less same.

The average number of hands per bunch and total number of fingers in bunch, number of suckers produced increased with increasing levels of S from first to second. Average finger weight was found to be enhanced with increasing levels of S and B and decreased with increasing levels of Ca and Mg. Decrease in average fruit weight may be due to increasing finger number and yield which means the partition of assimilates into a high number of fruit and consequently reduction in individual finger weight and other related characters.

Application of Ca would have also created a favorable soil environment by reducing acidity and it may also have protected the root plasma membrane from the deleterious effects of H^+ (Epstein and Bloom, 2005). Besides, the role played by Ca as a regulator ion in the translocation of carbohydrates through its effect on cells and cell wall (Bennett, 1993) might have lead to the higher fruit yield. Studies conducted by Nambiar *et al.* (1978) on the effect of graded doses of lime on growth and yield of banana var. Zanzibar at Kannara also reported that application of lime in graded dose contributed significantly for an increase in bunch weight and number of hands and number of fingers. Similar results were also reported by Moreno (1999) and Yang *et al.* (2010b).

Magnesium fertilization would have exerted positive effect on chlorophyll structure, enzyme activities, protein synthesis, carbon fixation and phosphate metabolism which would have lead to better growth and higher yield. A Similar result of yield enhancement in banana as a result of Mg application was also obtained by Mostafa *et al.* (2007).

Mostafa and Kader (2006) also found that fruit yield was increased in banana by increasing S application. The yield increase could be attributed largely to higher bunch weight, more number of hands per bunch and higher number of fingers. These results are in harmony with those obtained by Hedge (1988); Turner *et al.,* 1989; Kader *et al.,* 1990; and Hosam (2002).

The increase in yield by boron application can also be credited to the positive effect of boron on increasing the rates of carbohydrate and RNA metabolism (Parr and Loughman, 1983). The increase in fruit weight with the sprays of borax might be also due its involvement in hormonal metabolism, increase in cell division and expansion of cell. Yield enhancement in banana as a result of B application was also reported by Moreira (2011).

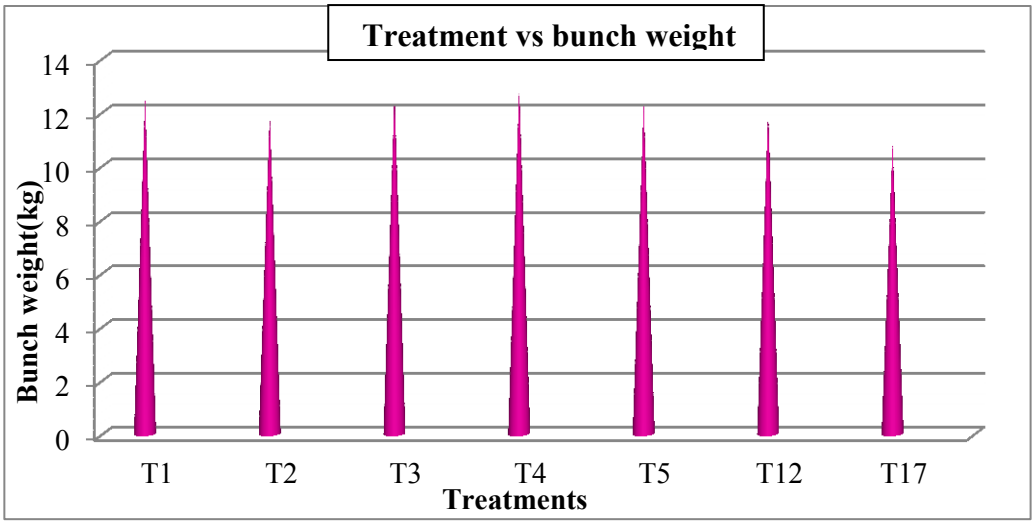

Figure 2: Effect of selected treatments on bunch weight

5.2.3. Effect of fruit characteristics

In fact, in high value crops like banana, quality standards have become the most important factor influencing monetary yield and farmer's income. Any management system should aim to produce quality fruits, besides maximizing productivity (Kumar and Kumar, 2007).

In the present study, it is found that the soil application of secondary nutrients and foliar application of boron on fruit characteristics of banana cv. Nendran indicated that treatment effects on quality parameters like titrable acidity and vitamin C content was significant whereas in TSS content there was no significant influence of treatments. The reducing sugar content and pulp peel ratio was also found to be enhanced due to treatment application.

Treatment application of secondary nutrients and boron exhibited significant differences in the titrable acidity percentage of the fruits. Figure 3 shows the effect of different levels of nutrients on titrable acidity of ripened fruits. A reduction in acidity is usually preferred and here it is found that the treatments have reduced the acidity percentage of the fruits compared to that of the control. The reduction in acidity might be due to more accumulation of sugars in the fruit (Ningavva *et al.,* 2014). Similar results were obtained by Patel *et al.,* 2010. The application of calcium compound might have reduced the acidity in fruits since it neutralizes or precipitates certain organic acids, which are derived in the metabolic activity of plants and/or used in the respiratory process as a substrate. Similar findings were also reported by Tripati and Shukla (2011) in aonla. Salama *et al.,* 2014 also reported that magnesium sulphate application reduced total acidity of date palm.

Ascorbic acid content (vitamin C) of the fruits exhibited significant differences among the treatments. Figure 4 shows the effect of different levels of nutrient on the vitamin C content of the fruits. Increasing levels of Ca and Mg have shown to improve the ascorbic acid content in ripened fruits. This increase in ascorbic acid content with calcium application may be due to uninterrupted synthesis of its precursor like glucose-6-phosphate during conversion of starch into various sugars and low rate of its oxidation. Similar results were also obtained by Singh *et al.* (1979) in peach and Tripathi and Shukla (2011) in aonla. Higher level of ascorbic acid may be also due to the application of boron as higher content of ascorbic acid are synthesized from sugar. Almost similar results were also reported by Kar *et al.,* 2002 in pineapple and Bhatt *et al.,* 2012 in mango.

Figure 5 shows the effect of different levels of nutrients on pulp peel ration. The maximum amount of pulp content might be due to foliar application boron which might have made rapid synthesis of metabolites particularly carbohydrates and their translocation to the fruits causing relatively greater pulp

content. This can also be possible as the complexes of polyhydroxy compounds with B can facilitate transport of carbohydrate within phloem tissue (Hewitt, 1963). Similar results were also reported by Ningavva *et al*., 2014 in banana and Singh *et al*., 2012 in aonla. Salama *et al.,* 2014 also reported that magnesium sulphate application enhanced the pulp weight of date palm. Similar results of increase in pulp: peel ratio of banana due to S application have also been reported by Kumar and Kumar in Neypoovan (2007) and Robusta (2008) cultivars.

Application of secondary nutrients and boron has found to bring beneficial effect on reducing sugar content of the fruits as compared to that of control. Figure 6 shows the effect of nutrient levels on reducing sugar content. The improvement in reducing sugar might be due to formation and translocation of carbohydrate which improves the fruit quality as reported by Pathak and Mitra (2008). Increase in sugar content might be due to the role of calcium in activation of the amylase enzyme which is responsible for the conversion of starch into sugar on ripening (Tripathi and Shukla, 2011). Mg supply might have enhanced the phloem transport of sugars to from the source (leaf) to sink (fruit). Similar results were also given by Cakmak (1994). Enhancement in the reducing sugar content may also be due to the role played by SO_4^{2-} ions as it favors the activity of anabolic enzymes, resulting in accumulation of highly polymerized carbohydrates (starch), which would have subsequently disintegrated into sugars on ripening (Kumar and Kumar, 2008). Influence of boron in translocation of sugars can also be accounted for the increase in sugar content since B expedites sugar transport across the membrane by a temporary formation of sugar borate complex (Gauch and Dugger, 1953).

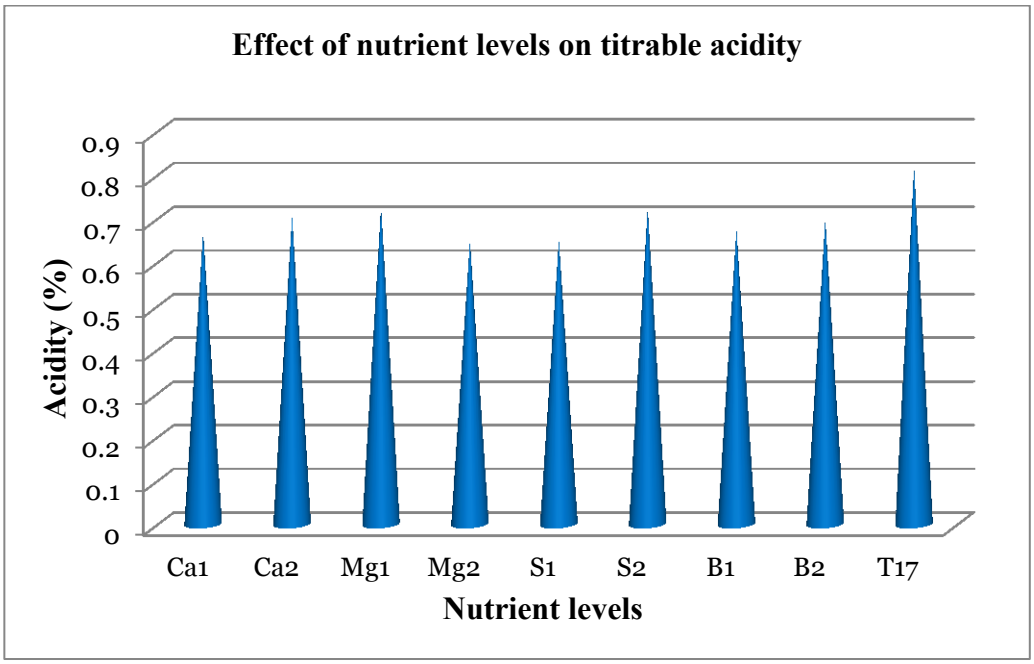

Figure 3: Effect of secondary nutrients and boron on titrable acidity of ripened banana fruit

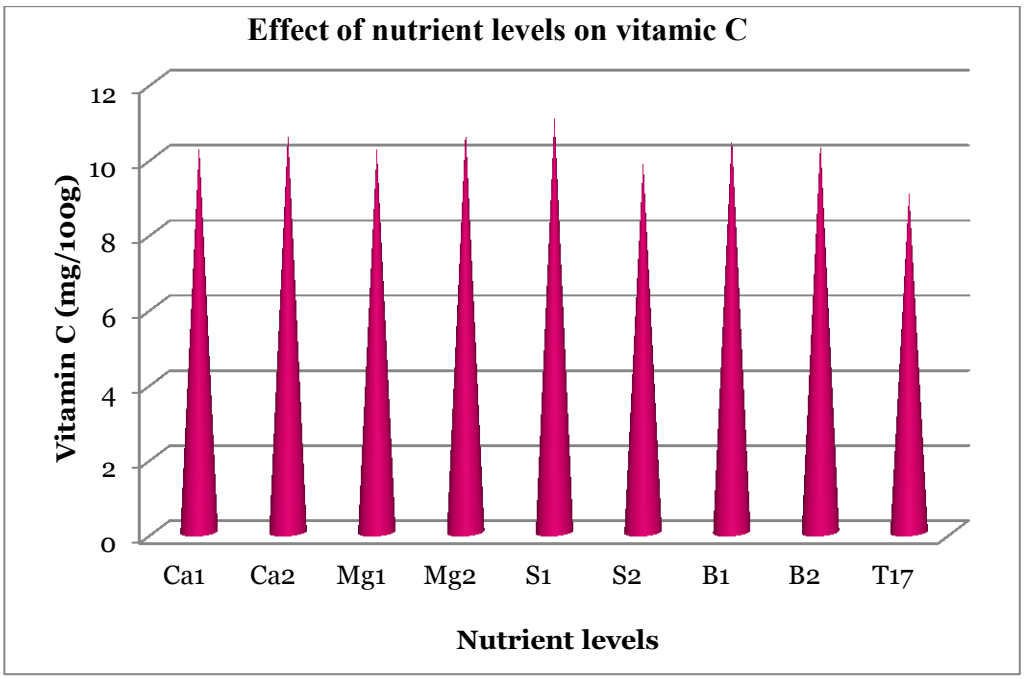

Figure 4: Effect of secondary nutrients and boron on ascorbic acid (vitamin C) content of ripened banana fruit

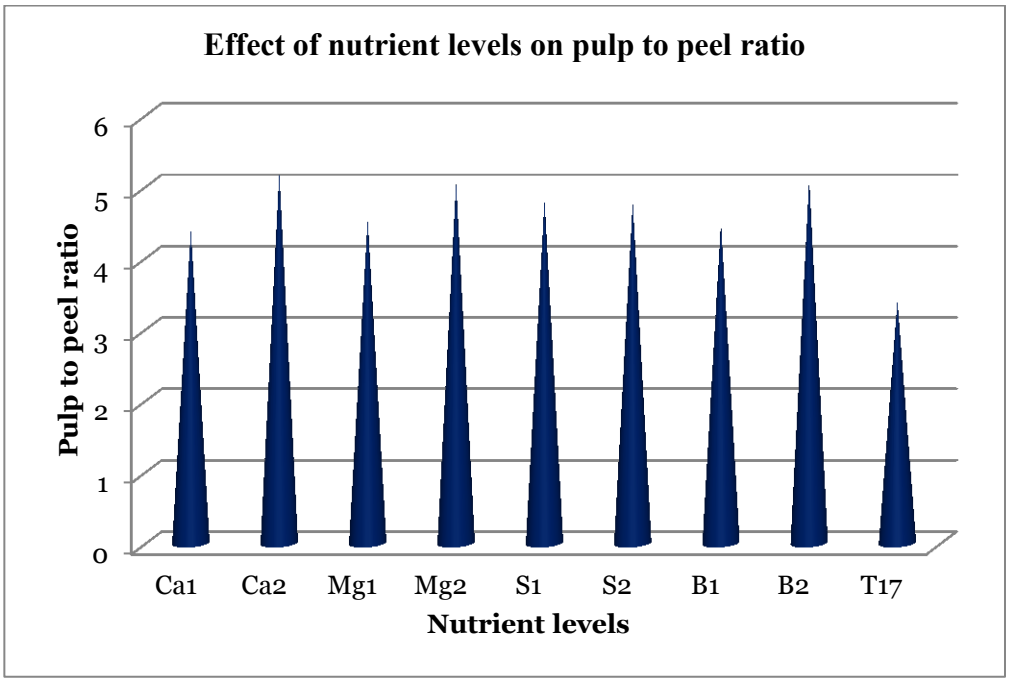

Figure 5: Effect of secondary nutrients and boron on pulp peel ratio of ripened banana fruit

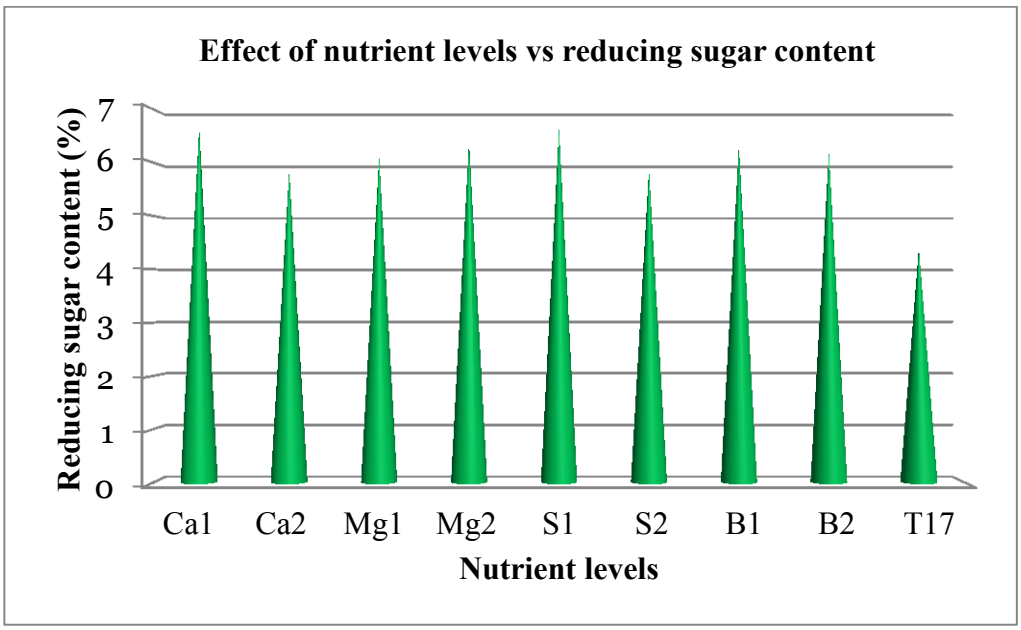

Figure 6: Effect of secondary nutrients and boron on reducing sugar content of ripened banana fruit

5.2.4. Effect on soil nutrient status

The effect of secondary nutrients and boron application on the soil nutrient status were studied and found that N, K, Ca, Mg, S, Fe, Mn, B and pH was found to be non significant. Eventhen, there was significant effect of treatments on EC, P, Cu and Zn of soil.

There was no significant effect of treatment application on soil pH. Eventhen, average pH of the treatment plots were higher than that of control plot, which in turn can enhance the nutrient availability and can bring about a better soil environment for plant growth compared to that of the control plots. This may be due to be due to the beneficial effect of Ca and Mg in increasing the soil pH by reducing the acidity whereas S application in certain soils has been reported to reduce pH levels and to increase SO_4^{2-} content (Lopez *et al*., 1999; Stamford *et al*., 2002). Excess uptake of anions over cations leads to the net removal of protons in the soil and cause an increase in soil pH. In contrast, excessive uptake of cations is charge balanced by a net-release of protons and consequently leads to soil acidification (Dilmaghani *et al.,* 2012). Cui *et al.* (2004) reported that the elemental sulfur acidified the soil. Mostafa and Kader (2006) also found the decrease in soil pH in alkaline soils due to S application.

Electrical conductivity of the soils exhibited significant differences among the treatments and average EC of the treatment plots were higher than that of control plot. It might be due to the increase in the soluble salt content of the soil as a result of treatment application. Also, increasing levels of Ca, Mg and S from first to second have shown to decrease the EC whereas for B it was increased.

Organic carbon content of the soils was not influenced significantly by the treatment application. On an average, OC content of treatments were found to be higher than for control. Increasing levels of Mg was found to enhance the OC whereas other nutrients decreased the OC content.

Nitrogen content of the soil was not significantly affected by the treatment application. There was significant difference among treatments regarding P content. Increasing levels of Ca decreased the P levels in soil whereas applications of Mg, S and B have found to enhance the P levels. K levels in the soil increased with increasing levels of Ca, Mg and B whereas it decreased with increasing levels of S. Murovhi (2013) in Valencia orange also reported that, even though non significant, increasing levels of S reduced the K content and increased the P content in soils. Besharati *et al*. (2000) also indicated that sulfur significantly caused an increase in the availability of phosphorus.

Calcium and Mg status of the soils were not found to be significantly influenced by the treatment applications. Magnesium content of the soils increased with application of increased levels of Ca, Mg, S and B. Sulphur content of the soils was also found to be non significant, eventhen S status in the treatment applied plots were higher than than of control. Non significance of secondary nutrients might be due to the increased plant uptake and leaching losses. Sims and Ellis (1983) also cited that under humid tropical climatic conditions, soils dominated by kaolinitic clays readily leaches out Ca^{2+}.

Cu content of the soils exhibited significant differences between the treatments. Increasing levels of Ca slightly increased the Cu content whereas increasing levels of Mg, S and B decreased the Cu content. When Zn status is considered, significant differences were observed between the treatments. Increasing levels of Ca and Mg decreased the Zn content whereas it has increased with increasing S and B applications.

Mn content of soil decreased with increasing levels of Ca and Mg whereas it increased slightly with increasing B and at the same time S have no effect on Mn content. Thus one of the benefits of Ca and Mg could be reducing the levels of Mn if it goes to toxic levels. Silva *et al.,* 2006 also reported the beneficial effect of liming in reducing the soluble levels of Mn in soil. Likewise, Fe content of the soils decreased with increasing levels of Ca and B whereas it

showed a very slight increase with increasing Mg and S levels. This shows the beneficial effect of treatments in reducing the toxic levels of Fe and Mn in the soils.

B levels in the soil were found to be higher in the soils which received foliar borax sprays. The contribution of foliar fertilization increasing the content in the soil may be due to the washing and by draining the fertilizer solution from the leaves to the soil surface (Nomura *et al.,* 2011).

5.2.5. Effect of leaf nutrient status

The effect of secondary nutrients and boron showed significant differences in the leaf nutrient status of Ca, Mg, S, B, Cu and Fe.

Treatment application caused a slight increase in the N and P content of leaves compared to control. Similar results were also obtained by Mostafa *et al.* (2007) from Egypt as a result of magnesium application and by Mustafa and Kader (2006) as a result of S application in banana. K content of the leaves of treatment plots was slightly lower than the control eventhough there was no significant differences. Similar finding was also obtained by Mostafa *et al.* (2007) as a result of magnesium application in banana.

Regarding the secondary nutrient content of the leaves there was significant differences between the treatments and the control. Concentrations of Ca and Mg in the leaves were found to be higher than that of control. Increasing levels of Ca and B from first to second level was found to increase the Ca content in leaves whereas increasing levels of Mg and S decreased the Ca content. Silva *et al.,* 2006 also reported the increase in Ca content in the leaves of pineapple fertilized with lime.

Increasing levels of all the nutrients except S from first to second reduced the Mg content of leaves. Eventhen the Mg status of the treatments were better than that of control. Mostafa *et al.* (2007) also reported increase in the leaf Mg content of banana as a result of Mg fertilization. Similar results were also reported by Fawzi *et al.* (2010) in pear; Ahmed *et al.* (2012) on Washington

navel orange; Salama *et al.* (2014) in date palm. To obtain high yield and good quality fruit, the banana plant requires a large quantity of K. Large uptake of K may lead to translocation of Mg toward the fruit and storage tissues, decreasing the Mg concentration throughout the plant. Similar results was also obtained by Silva (2013) who found that reduction of Mg leaf content in a quadratic manner with increase in the doses of Mg applied to the soil in the first cycle of banana. Increase in the K availability may have reduced the leaf Mg content. Similar results were found by Silva *et al.* (2008) and Ganeshamurthy *et al.* (2011), who observed that with increase in the K levels in the soil, the leaf Mg content decreased. Decrease in the Mg content in the leaf tissues may be attributed to the competitive effect among these nutrient ions (Malavolta, 2006).

Increasing the nutrient concentrations of secondary nutrients and B from first to second level have increased the S content of the leaves. Similar findings of increase in the S content of the leaves as result of S application was also reported by Mostafa and Kader (2007) in banana, Hasan *et. al.* (2000) and Deen (2002). In general the lesser S content in leaves might be due to the reduced sulphur uptake after shooting and the sulphur needed for further growth might have derived from the leaves and pseudostem as reported by Walmsley and Twyford (1976b).

Significant differences were noticed in the Mn uptake among the treatments. However, very high levels of Mn were observed in banana leaves. Turner and Barkus (1973) also reported the high manganese concentration in the leaves as a special character of banana. Average Mn content of the treatments was found to be lower than that of control. A concentration upto 2200 ppm is considered as optimum for banana whereas above which it is high under subtropical conditions as reported by Turner (1983). Increasing levels of Ca from first to second reduced the Mn content in the leaf by around 1000 ppm whereas the Mn content in control it was very high. Hue and Mai (2002) also found that high calcium can reduce manganese uptake. Increasing levels of Ca, Mg and B was found to reduce the Mn concentration in the leaves whereas

increasing S levels increased the Mn levels. Turner and Barkus (1983) pointed out that in banana uptake of Mn was not affected by the presence of K or Mg.

High Mn supply in the soil may have depressed the uptake rate of Mg and Ca and greatly increased the uptake of Mn. A feature was the tolerance of the banana to high concentrations of Mn in the soil solution. Despite a sevenfold increase in Mn content in the plant, dry matter was not significantly reduced. However, high Mn could be a problem in soils with marginal supplies of Mg, Ca and Zn because of its effect on the uptake of these elements. High Mn reduced the ratio of the concentration of Mg in the plant to the concentration in the solution around the roots and therefore its effect on Mg must be at the root surface (Turner and Barkus, 1983). He also reported that K and Mn non competitively inhibited Mg uptake.

Average Fe concentration of leaves in treatments was found to be higher than that of control but at the same time increasing levels of Ca, Mg, S and B reduced the uptake of Fe in plants. Oxidation of elemental S can create an acidic environment in the root vicinity which will facilitate reduction of Fe^{3+} to Fe^{2+} which is water soluble and more available and can raise the Fe content of leaves and it might have lead to higher Fe uptake. Similar reports were also given by (Romheld, 1987). Murovhi (2013) also reported the reduction in Fe content with increasing levels of S in orange.

Average Cu content of the leaves in treatments were lower than that of control. Increasing levels of Ca, S and B increased the Cu content whereas increasing Mg levels decreased the Cu content of leaves. Turner and Barkus (1983) also reported that there was no large overall effect of Mg supply on the uptake of elements other than Cu. Mg increased Cu uptake when K was low but had little effect when it was sufficient as reported by the authors.

Average Zn concentration in the treatment applied leaves was higher than that of control. Increasing levels of Ca and B was found to increase the Zn status of leaves whereas Mg and S reduced it.

Significant difference in the B concentration of the leaves was noticed. Increasing concentration of B from first to second levels increased the B levels in leaves. Moreira *et al.,* 2011 also reported that B application rates caused significant and linearly increase in B foliar concentrations in banana.

Benefit cost ratio of the best treatment (T_4) extrapolated from twelve plants is found to be 2.85 (@ Rs 35/kg of fruit). So this shows that the treatment is promising higher returns to the farmers.

Summary

6. SUMMARY

The salient findings of the present study entitled "Management of calcium, magnesium, sulphur and boron in TC banana (*Musa* spp.) var. Nendran" are summarized in this chapter.

Investigations were carried out at College of Agriculture, Padannakkad and Regional Agricultural Research Station farm of Nileshwar during December 2013 to January 2015, with the objective to study the effect of application of calcium, magnesium, sulphur and boron on hardening of tissue culture plants and application of these nutrients in the field conditions of TC banana var. Nendran. The whole study consisted of two parts – hardening studies and field experiment.

The hardening studies were conducted in the poly house of College of Agriculture, Padannakkad, Kerala with TC banana plants of uniform size derived from same culture. The experiment was carried out in completely randomized design with two levels each of calcium (75 and 150 ppm), magnesium (25 and 50 ppm), sulphur (25 and 50 ppm) as soil application and boron (0.25 and 0.5 %) as foliar sprays. There were sixteen treatment combinations and one control with three replications each. Four plants were maintained in each of the replications. The potting mixture was prepared uniformly with 1:1:1 ratio of soil, sand, cowdung and then it was fortified using the various treatment combinations of calcium, magnesium, sulphur and the plants were raised in polybags for a period of two months. Boron application in the form of foliar spray at the above two levels of concentration was carried out one spray immediately after planting and the second at one month after planting. Significant differences among the treatments were observed in the biometric characteristics of the crop. T_{10} (150 ppm Ca, 25 ppm Mg, 25 ppm S and 0.5 % B) recorded maximum number of leaves, leaf length, leaf breadth and plant height.

The field experiment was carried out at Regional Agricultural Research Station farm of Nileshwar to study the effect of application of calcium, magnesium, sulphur and boron in tissue culture banana var. Nendran. Two

hundred and four hardened tissue culture banana plantlets were planted in randomized block design with seventeen treatments and three replications such that four plants were maintained in each replication. Nitrogen, Phosphorus, Potassium application and other cultural practices were uniformly followed for all plants as per Package of Practices, KAU (2011).

The treatments consisted of soil application of two levels of calcium (75 and 150 g Ca/plant), magnesium (25 and 50 g/plant) and sulphur (25 and 50 g/plant) at second and fourth month after planting along with two levels of boron (0.25 and 0.5 %) foliar sprays at first, second, fourth month after planting and one after bunch emergence.

The results of the field experiment showed that treatment application significantly enhanced the bunch weight, number of hands and number of leaves on the plant at the time of bunch emergence. The highest bunch weight and number of fingers were recorded in T_4 (75 g Ca + 25 g Mg + 50 g S + 0.5 % B) with 12.97 kg as against 10.34 kg in control. Maximum weight of male bud was found in T_6 (75 g Ca + 50 g Mg + 25 g S + 0.5 % B), number of leaves present at the time of bunch emergence and plant height was found to be highest with T_{12} (150 g Ca + 25 g Mg + 50 g S + 0.5 % B), number of hands in T_{11} (150 g Ca + 25 g Mg + 50 g S + 0.25 % B), pseudostem girth at 30 cm and average number of suckers produced were highest in T_3 (75 g Ca + 25 g Mg + 50 g S + 0.25 % B), average weight of fingers were highest in T_{10} (150 g Ca + 25 g Mg + 25 g S + 0.5 % B), average finger length was in T_3 (75 g Ca + 25 g Mg + 50 g S + 0.25 % B), and girth in T_5 (75 g Ca + 50 g Mg + 25 g S + 0.25 % B). T_7 (75 g Ca + 50 g Mg + 50 g S + 0.25 % B) produced bunch in the shortest time of 170 days whereas T_9 (150 g Ca + 25 g Mg + 25 g S + 0.25 % B) took minimum days (260) to harvest. Eventhough statistically non significant, control plants shown greater degree of sigatoka leaf spot infestation compared to treatments.

Fruit characteristics like TSS, titrable acidity, vitamin C content, reducing sugar content and pulp to peel ratio were studied. Of these, titrable acidity, vitamin C content, reducing sugar content and pulp to peel ratio showed

significant differences among the treatments. Reducing sugar content and pulp to peel ratio of all the treatments were found to be superior to the control. Among the treatments, highest vitamin C content was found in T_5 (75 g Ca + 50 g Mg + 25 g S + 0.25 % B) with 15.39 mg/100g of fruit. Lesser acidity indicates better quality and average titrable acidity recorded in the treatment applied fruits was found to be lowest in T_2 (75 g Ca + 25 g Mg + 25 g S + 0.5 % B). Maximum TSS content (30.17 ^{0}brix) and reducing sugar percentage (8.3 %) was recorded in T_6 (75 g Ca + 50 g Mg + 25 g S + 0.5 % B). Among the treatments, lowest pulp peel ratio was in control (3.38) and highest ratio of 6.77 was observed in T_{16} (150 g Ca + 50 g Mg + 50 g S + 0.5 % B).

The effect of secondary nutrients and boron application on soil nutrient status of N, K, Ca, Mg, S, Fe, Mn, B and pH was found to be non significant. Eventhen, there was significant effect of treatments on EC, P, Cu and Zn of soil.

Eventhough there was no significant differences among treatments, average pH values of the soil in treatment plots were higher than that of control plot. Highest pH (5.06) was found in T_{16} which received second levels of Ca, Mg, S and B. Electrical conductivity of the soils exhibited significant differences among the treatments and average EC of the treatment plots were higher than that of control plot. Highest EC was found in T_4 (75 g Ca + 25 g Mg + 50 g S + 0.5 % B) while lowest was in T_{16} (150 g Ca + 50 g Mg + 50 g S + 0.5 % B). Organic carbon content of the soils was not influenced significantly by the treatment application. On an average, OC content of treatments were found to be higher than for control. Highest OC was found in T_5 (75 g Ca + 50 g Mg + 25 g S + 0.25 % B).

Available N content of the soil was not significantly affected by the treatment application. Highest available N was recorded in T_7. Significant difference among soil P content was recorded. Increasing levels of Ca decreased the P levels in soil whereas applications of Mg, S and B have found to enhance the P levels. K levels in the soil increased with increasing levels of Ca, Mg and B whereas it decreased with increasing levels of S. Highest available P and

available K were in T_{14} (150 g Ca + 50 g Mg + 25 g S + 0.5 % B) and T_6 (75g Ca + 50g Mg + 25g S + 0.5% B), respectively.

Ca status of the soils was not found to be significantly influenced the treatment applications. T_9 (150 g Ca + 25 g Mg + 25 g S + 0.25 % B) and T_6 (75 g Ca + 50 g Mg + 25 g S + 0.5 % B) recorded highest Ca content. Mg content of the soils increased with application of increasing levels of Ca, Mg, S and B. Mg content was highest in T_7 (75 g Ca + 50 g Mg + 50 g S + 0.25 % B). S content was highest in T_{10} (150 g Ca + 25 g Mg + 25 g S + 0.5 % B).

Among the micronutrients Cu and Zn showed significant differences among treatments with respect to their soil content. Increasing levels of Ca slightly increased the Cu content whereas increasing levels of Mg, S and B decreased the Cu content. Highest Cu was found in T_{13} (150 g Ca + 50 g Mg + 25 g S + 0.25 % B). Increasing levels of Ca and Mg decreased the Zn content whereas it was increased with increasing S and B content. Highest soil Zn content was recorded in T_8 (75 g Ca + 50 g Mg + 50 g S + 0.5 % B). Mn content of soil decreased with increasing levels of Ca and Mg whereas it increased slightly with increasing B levels and at the same time S have no effect on Mn content. Mn in T_4 (75 g Ca + 25 g Mg + 50 g S + 0.5 % B) recorded highest value. Likewise, Fe content of the soils decreased with increasing levels of Ca and B whereas it was slightly increased with increasing S and B levels. Fe was found to be highest in T_7 (75 g Ca + 50 g Mg + 50 g S + 0.25 % B). B concentration in the soils was found to be higher in the soils where the plants were sprayed with foliar borax sprays. Highest B content was in T_{16} (150 g Ca + 50 g Mg + 50 g S + 0.5 % B).

As the leaf nutrient concentration of banana is concerned significant influence of treatment application of secondary nutrients and boron were found in Ca, Mg, S, B, Fe and Mn status of the leaves. Treatment application caused a slight increase in the N and P content of leaves compared to control. Highest N content was found in T_5 and P content in T_9. K content of the leaves of treatment

plots was slightly lower than the control even though there was no significant difference.

Regarding the secondary nutrient contents of the leaves there were significant differences between the treatments and the control. Concentrations of Ca, Mg, and S in the leaves were found to be higher than that of control. Increasing levels of Ca and B from first to second level was found to increase the Ca content in leaves. Highest Ca content was found in T_2. Increasing levels of all the nutrients from first to second reduced the Mg content of leaves. Highest content was found in T_7. Increasing levels of S increased the S content in leaves and the highest S content was found in T_{14}.

Average Fe concentration of leaves in treatments was found to be higher than that of control but at the same time increasing levels of Ca, Mg, S and B reduced the uptake of Fe by plants as reflected in leaf concentration. Average Cu content of the leaves in treatments were lower than that of control. Average Mn content of the treatments was found to be lower than that of control. Average Zn concentration in the treatment applied leaves was higher than that of control. Increasing levels of Ca and B was found to increase the Zn status of leaves whereas Mg and S reduced it. Even though there was no significant differences in the B concentration of the leaves, increasing concentration of B from first to second levels increased the B levels in leaves. Highest Fe, Cu, Zn, Mn, and B were found in T_2, T_{17}, T_{10}, T_{17}, and T_{16} respectively.

Benefit cost ratio of the best treatment (T_4) extrapolated from twelve plants is found to be 2.85 (@ Rs 35/kg of fruit). So this shows that the treatment is promising higher returns to the farmers.

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MANAGEMENT OF CALCIUM, MAGNESIUM, SULPHUR AND BORON IN TC BANANA (*Musa* spp.) var. NENDRAN

by

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ABSTRACT

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ABSTRACT

The experiment entitled "Management of calcium, magnesium, sulphur and boron in TC banana (*Musa* spp.) var. Nendran" was carried out with the objective to study the effect of soil application of calcium, magnesium, sulphur and foliar application of boron on hardening of tissue culture (TC) plants and the application of these nutrients in the field. The investigations were carried out at College of Agriculture (COA), Padannakkad and RARS farm at Nileshwar during December 2013 to January 2015. The whole study consisted of two parts– hardening studies and field experiment.

The hardening studies were conducted in the mist chamber at COA, Padannakkad with TC banana plants of uniform age and size derived from same culture. The experiment was carried out in CRD with two levels each of calcium (75 and 150 ppm), magnesium (25 and 50 ppm), sulphur (25 and 50 ppm) and boron (0.25 and 0.5 %). There were sixteen treatment combinations and one control with three replications. Four plants were maintained in each of the replications. The potting mixture was prepared uniformly and then it was fortified using the various treatment combinations of calcium, magnesium, sulphur and the plants were raised in polybags for a period of two months. Boron application in the form of foliar spray at two levels of concentration was carried out immediately after planting and one month after planting. Significant differences among the treatments were observed in the biometric characteristics of the plants. T_{10} (150 ppm Ca, 25 ppm Mg, 25 ppm S and 0.5 % B) recorded maximum number of leaves, leaf length, leaf breadth and plant height.

These two hundred and four hardened tissue culture banana plants were planted at RARS farm, Nileshwar in RBD with seventeen treatments and three replications. Four plants were maintained in each plot. Major nutrients *viz.* N, P, K application and other cultural practices were uniformly followed for all plants as per POP, KAU (2011). The treatments consisted of soil application of two levels of calcium (75 and 150 g Ca/plant), magnesium (25 and 50 g/plant) and sulphur (25 and 50 g/plant) at second and fourth month after planting along with

two levels of boron (0.25 and 0.5 %) foliar sprays at first, second, fourth month after planting and one spray after bunch emergence. Observations of vegetative characters were taken at the time of bunch emergence.

The results of the field experiment revealed that among the vegetative characters, number of leaves showed significant differences among the treatment. Highest leaf number and plant height were found in T_{12} whereas pseudostem girth and number of suckers produced were highest in T_3. Among the yield characteristics bunch weight, number of hands, number of fingers, average weight of fingers and average number of suckers showed significant difference. The highest bunch weight and number of fingers were recorded in T_4 (75 g Ca + 25 g Mg + 50 g S + 0.5% B) with 12.97 kg as against 10.34 kg in control whereas number of hands was highest in T_{11}. Among the finger characteristics, average weight of finger was highest in T_{10}, length and girth in T_3 and T_5 respectively. T_3 produced maximum number of suckers at the time of harvest. The time span taken from planting to bunch emergence and harvest were non significant. Disease (sigatoka leaf spot) incidence eventhough non significant, compared to control plots the treated plots registered lower incidence.

Fruit characteristics like TSS, titrable acidity, vitamin C content, reducing sugar content and pulp to peel ratio were studied. Of these, titrable acidity, vitamin C content, reducing sugar content and pulp to peel ratio showed significant differences among the treatments. Reducing sugar content and pulp to peel ratio of all the treatments were found to be superior to the control. Among the treatments, highest vitamin C content was found in T_5. Average titrable acidity was found to be lowest in T_2 and highest was in control. Maximum TSS content and reducing sugar percentage was recorded in T_6. Among the treatments, lowest pulp peel ratio was in control and highest ratio of was observed in T_{16}.

After the harvest, the effect of these treatments on soil nutrient availability was studied. The results showed that EC, P, Cu and Zn status were

significant. Similarly, leaf nutrient analysis at the time of harvest revealed that Ca, Mg, S, B, Fe and Mn showed significant differences among the treatments.

Both the hardening studies and field experiment indicated the beneficial effect of secondary nutrients and boron on TC banana. T_{10} (150 ppm Ca, 25 ppm Mg, 25 ppm S and 0.5% B) and T_4 (75 g Ca + 25 g Mg + 50 g S + 0.5% B) were found to be performing well under hardening and field conditions, respectively.

Benefit cost ratio of the best treatment (T_4) extrapolated from twelve plants is found to be 2.85 (@ Rs 35/kg of fruit). So this shows that the treatment is promising higher returns to the farmers.