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Research Article

Agronomic biofortification of calcium in cabbage (*Brassica Oleracea var capitata)* applied with different sources of liming in Ca deficient acidic soil of Coonoor, The Nilgiris (Typic Dystropept)

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Abstract
The human body needs calcium (Ca) to maintain strong bones and teeth and to build a strong structure, helping muscles contract and playing a crucial role in the structural and signalling process. However, low calcium consumption in the diet has related to a variety of disorders in humans, which can have long-term health repercussions. Therefore, this study aimed to evaluate the Ca biofortification capacity of cabbage (*Brassica oleracea var capitata*) supplied with different Ca-supplying inorganic fertilizer sources at various fixed levels based on soil liming potential grown in open field conditions where four hybrids of cabbage grown in Ca deficient acidic soil. Ca applied as Limestone ($CaCO_3$) (150% and 175% liming potential) and Dolomitic limestone [$CaMg(CO_3)_2$]150% liming potential yield high Ca content in cabbage head and foliage (61.3 mg 100 g^{-1}), high glucosinolates content (53.12 mg 100 g^{-1}) and lower oxalate(0.31 mg 100 g^{-1}) that produced firmer head as compared with Ca untreated control which also promoted high market value for Ca biofortified ones. On the other hand, Ca addition leads to lower Fe and Mg content in the cabbage tissues due to an antagonistic effect. All four hybrids of cabbage studied using the agronomic method of biofortification significantly($p \leq 0.05$) improved Ca enrichment (20% more compared to control) without showing any toxicity symptoms making possibility to obtain Ca biofortified cabbage in acidic soil of a hilly ecosystem by application of liming.

Keywords: Acidic soil, Biofortification, Cabbage, Calcium, Liming potential

INTRODUCTION

Calcium is an essential nutrient having a specific role in human metabolism. Calcium is directly linked with bone health, where lower Ca in the diet leads to low bone mineral density and is epidemiologically linked with other risks such as osteoporosis, bone deformity in infants, rickets, and hypertension (Harinarayan *et al.,* 2021). Agronomic biofortification is the widely adopted strategy to surpass mineral malnutrition in developing countries, where agronomic biofortification focuses on the population's staple diet. If the concentration of a particular mineral can be increased in staple food, then the target population vulnerable to mineral malnutrition gets delivered to certain minerals without changing food habits (White and Broadley, 2003). In this context, increasing Ca concentration in food crops by biofortification dramatically increases the Ca in the human diet. Calcium concentration of food shows a wide variation that depends on the amount of anti-nutritional factors present. Also, with the increasing vegan culture, popularity of the salad diet, and lactose intolerance, there is vital to find high Ca supplementary foods other than dairy products. Calcium accumulation and biofortification in edible parts of crops are mainly based on solubilisation, application rate, soil type, time of application, and tissue accumulation (Dayod *et al.,* 2010; Pessoa *et al.,* 2021)

In soil, Ca is the most plentiful nutrient, the dominant cation on the soil exchange complex in many soil types except acid soils. Uptake of Calcium to plants has significant functions inside the plant system, such as signal transduction agent (Wei *et al.,* 2017), maintaining cell wall integrity by forming calcium pectate (Hocking et al., 2016), promoting root hair growth, aiding in meristematic tissue growth in the stem (Bonomelli *et al.,* 2019), a cofactor in plant enzymes such as ATP and phospholipids catabolism, acts as a secondary messenger in plant signalling networks in a variety of developmental, stress-related and physiological process. Acidic soil environments are the most susceptible to calcium deficiency, where most of the exchange sites are filled with high levels of extractable aluminium and manganese. Further acid soils have the following characteristics: high phosphate sorption capacity, rich in Si, Fe, K, and Na, and poor in calcium, magnesium, and molybdenum (Behera *et al.,* 2015).

Amending acid soils with Ca amendments is the common agronomic practice to reduce Al- toxicity and acidity and restore calcium availability to plants (Muindi et al., 2015). Also, these liming practices are positively correlated with the quality of economic parts, crop yield, increase in total dry matter content of the crop, and effect on reducing Al- toxicity to plants (Buni, 2015). Cabbage is an important Cole crop grown in acidic soil conditions and thrives well in temperate conditions. Cabbage is highly demanding among the other brassica crops and has high calcium content in its foliage and head. Also, cabbage has low oxalate and phytate content compared to other hill crops. These oxalate and phytate form complexation with Ca, resulting in low bioavailability that consequences dietary deficiency diseases. In plants, Ca is absorbed as a divalent cation in the region of lateral shoot initiation, translocates primarily through the xylem along with transpiration, and accumulate more in leafy vegetables compared to phloem fed organs such as fruits and tubers, which opens a target for calcium biofortification (Demidchik *et al.*, 2018; Kerton *et al.*, 2009). The significant points to be considered in calcium biofortification are increasing the addition of Ca to cells, crop uptake, removing the anti-nutritional compound that makes Ca unavailable, and increasing the Ca storage at cellular and tissue level (Buturi *et al.,* 2021). Taking the above content into account, agronomic biofortification of cabbage with Ca has experimented with different calcium sources applied in various levels of the liming potential of soil and as foliar spraying using four different hybrids of cabbage as test crops predominantly being cultivated in the Nilgiris biosphere. The main objective was to compare the variations in total Ca content, calcium uptake, the differential behaviour of sources and levels, and the concentration of anti-nutrition in the cabbage plant (*Brassica olaracea var capitata*)

MATERIALS AND METHODS

Experimental layout

A micro plot experiment was conducted on four cabbage hybrids as a test crop in an open field condition of the experimental farm located at a State horticultural farm ($11^0$19' N, $76^0$48' E), The Nilgiris, India, between August and December 2021. Since hybrids are ruling as farmers' choices in this area, four hybrids are selected considering this aspect: Tekila, Quisto, Saint, and NBH Nova 50. Cabbage seeds were procured and disinfected by soaking in 1% $KMnO_4$ solution for 20 minutes before being sown into portrays that were thoroughly maintained in the controlled condition for 30 days and transplanted into field conditions where the seedlings were in 5-6 leaves. The spacing of 45 cm^2 was maintained between the seedlings, and the experiment was carried out in a Factorial randomized block design (FRBD) with three replicates. Calcium sources such as Limestone ($CaCO_3$), Ca- EDTA, dolomite [$CaMg(CO_3)_2$], and rock phosphate were used with three levels of the liming potential of soil, as 125%, 150%, and 175% were added, and 100% of liming potential was maintained as control. Based on the initial soil analysis, both control and treated plots were added with a recommended dose of NPK and micronutrient fertilizers. The Ca fertilizers such as $CaCO_3$, dolomitic Limestone, and Rock phosphate were applied as basal, and Ca-EDTA was applied as a foliar spray since it is toxic to plants at 30 days intervals. After applying calcium fertilizers, the soil was inverted back and irrigated at every interval to ensure that it was well combined and readily available to plants.

Initial soil analysis

To quantify initial soil parameters, nine samples collected from 15 to 30 cm depth in various parts of the experimental field were carefully processed, dried at 105^0C, and used as initial soil analysis. Soil reaction and electrical conductivity were measured in the 1:2.5 ratio suggested by (Jakson, 1964). Mechanical analysis was carried out to find the soil textural class procedure suggested by (Piper,1966). Physical parameters of soils such as bulk density, particle density, and porosity (%) were calculated. Organic carbon (Walkley and Black, 1934), Free $CaCO_3$, and CEC (Page *et al.,* 1982) are the initial chemical analysis done. Additionally, mineral nutrients such as available Nitrogen (Subbaiah, 1956), phosphorus, potassium (Jakson, 1964), micronutrients (Lindsay and Norvell,1978) and liming potential (Shoemaker *et al.,* 1961) were analysed (Table 1).

Physiological, yield, and quality measurements

The experimental plot was carefully monitored and recorded the physiological parameters such as plant height, and the normalized SPAD index was taken at 940 nm with a portable chlorophyll meter (SPAD 502, Minolta) at 30 days interval from transplanting to harvest stage in the last expanded leaf. Regarding yield attributes, total head yield and total plant biomass were calculated. In addition, the important quality parameters, such as ascorbic acid content (da Silva *et al.,* 2021), titrable acidity (Yoon *et al.,* 2006), and peroxidase content, were assayed using ABTS as substrate in UV-VIS spectrophotometer (Lab India UV-VIS 3092) (Joel *et al*., 2020; Posmyk *et al.,* 2009) were determined from the cabbage head tissues.

Plant oxalate and glucosinolates content

The oxalate content of the plant sample is determined by the titration method suggested by (Chemists and Horwitz 1975). The cabbage tissue is oven-dried at 45^0 C for 24 hours and then processed. To determine oxalate content, a 1g sample was weighed and added with 20 ml of 0.1M HCl to extract total oxalate. Another sample was treated with 20 ml of distilled water for extracting soluble oxalate. The beaker is kept in a water bath for 30 minutes, followed by filtering in Whatman No.1 filter paper. Then the filtrate is added with 0.5 ml of 5% $CaCl_2$ and subjected to a centrifugation process of 3500 rpm for 15 minutes to precipitate the calcium oxalate, and the supernatant is discarded. Then the calcium oxalate is washed with 2 ml of 0.35M NH_4OH and dissolved in 0.5 M H_2SO4. Then the dissolved solution is titrated with 0.1 M $KMnO_4$ till the light pink colour persists for about 15 sec. Insoluble oxalate content is obtained by separating the soluble oxalate content from total oxalate. Glucosinolates were analysed based on the procedure (Bell, Wagstaff, and chemistry 2014), involving grounded plant samples being oven-dried and treated with 70% methanol preheated at 70^0C and placed in a water bath for 20 min. Then the sample is centrifuged for five minutes at 3000 rpm, filtered, and the contents are analysed by Spectrophotometer.

Table 1. Initial soil analysis for the experimental field

Type of analysis	Parameters
pH $_{H2O}$	4.92
Conductivity	0.052
C-organic	0.72
Ca-Exchangeable [c mol (p+)kg^{-1}]	4.14
Mg- Exchangeable [c mol (p+)kg^{-1}]	1.70
N-available kg N ha^{-1}	218.50
P-Bray kg P_2O_5 ha^{-1}	28.11
K kg K_2O ha^{-1}	125.60
CEC [c mol (p+)kg^{-1}]	12.87
Texture	Sandy clay loam
Sand (%)	61.90
Silt (%)	9.23
Clay (%)	28.87
Bulk Density	1.32
DTPA-Zn mg kg^{-1}	2.32
DTPA-Fe mg kg^{-1}	5.76
DTPA-Cu mg kg^{-1}	2.88
DTPA-Mn mg kg^{-1}	112.43

Plant calcium and total Ca uptake

The calcium content in the cabbage tissue is analysed in Microwave plasma atomic emission spectroscopy (Agilent 4210 MP-AES) in the wavelength range of 396.84 nm. The plant samples are digested in the Borosil digestion chamber (KSC010) by adding 12 mL of 7 mol L^{-1} HNO_3 and 5 mL of H_2O_2 to 1g of sample from the edible portion cabbage. Then the digested solution is filtered and made up of 10 mL by adding distilled water and analysing the Ca content against a standard solution. The total Ca uptake was calculated using the parameters of dry matter production and calcium concentration in the head and foliage (da Silva *et al.,* 2021).

Inorganic ion content in plant tissue

To determine cationic nutrients (Na, K, Ca, and Mg), the cabbage sample was dried at 60^0 C in a hot air oven, followed by ashed in a muffle furnace at 550^0 C and digested with HCl of concentration 20 mL in the water bath about 30 min. The resultant solution was filtered to obtain a clear solution and analysed for K and Na in the Flame photometer (FP-902). The anionic nutrients, such as Nitrogen, were estimated by extracting the solution by sulphuric acid digestion and determined in Kjeldahl distillation. Phosphorus was estimated by digesting the sample in the tri-acid, followed by analysing the contents using the colorimetric method, where the measurement was done in a UV-VIS spectrophotometer (LabIndia-3092) (Page *et al.,* 1982). The micronutrients in the plant sample were extracted using nitroperchloric digestion and determined by Atomic absorption spectrophotometer.

Statistical analysis

Statistical analysis of the experimental data was carried out using the one-way ANOVA method to assess differences among the calcium sources, different varieties, and variation in levels, followed by a comparison of means by Duncan's multiple range tests (DMRT) and the critical difference were made at P=0.05 probability with the use of statistical software SAS package (Gomez and Gomez, 2010)

RESULTS AND DISCUSSION

Effect of treatments and levels on physiological and yield parameters

The initial pH of the soil was 4.92, which falls in acidic soil reaction. The analysis of soil liming potential shows that liming requirement to bring the desired pH for cabbage production was 6.4, which requires 6.4 t ha-1, calculated plot-wise. The highest head yield was recorded in the treatment that received Limestone and dolomite. The interaction effect revealed that the highest head yield was recorded in the application of dolomite with 175% liming potential in the hybrid Tekila (average 40.10 t ha^{-1}) compared with other treatments and hybrids is, followed by dolomite with $_{150\%\ liming\ potential.}$ These findings are similar to the experiment conducted with the cabbage with different lime fertilizers in acidic soil conditions (Zonayet and Ahmed, 2020). That could be attributed that lime application, more P desorption occurs into the soil solution, which is readily available to cabbage and cauliflower, thereby enhancing the yield (Nazrul and Shaheb, 2016). The highest plant biomass recorded in the plot fertilized with limestone$_{150LP\%}$ in the hybrid Saint with the mean value of 5.28 t ha^{-1}. The lowest biomass was recorded in the treatment that received Ca-EDTA at all levels where no significant difference was found between other calcium treatments and levels probably due to adequate supply of Ca did not influence dry matter content matches with the context of other authors (Durate *et al.,* 2019) who found that highest dry matter accumulation is Ca promotes rapid cell division during head initiation. These results are consistent with the findings of Lee *et al.* (2008), where high Ca in the acidic condition leads to more root hair proliferation and increases the root volume directly proportional to dry matter yield. Agronomic biofortification approaches did not reduce the yield as reported by the previous authors working on different crops with different nutrients such as Ca in leafy vegetables, I in cabbage and cowpea, Zn and Fe in Brassicae microgreens, Se in cabbage cultivars (D'Imperio *et al.,* 2016;Ojok *et al.,* 2019; Di Gioia *et al.,* 2019; de Almeida *et al.,* 2022; Prasad and Shivay, 2020) as it supplies the sufficient nutrient according to the crop needs.

Regarding chlorophyll content of different hybrids, high chlorophyll content was recorded in treatment received Limestone and dolomite as a calcium source. There was no significant difference between the plots that received rock phosphate and Ca-EDTA in colour parameters with different hybrids. Tekila showed a high amount of chlorophyll content among all four hybrids, and a visible difference was observed in the entire growth period. The visible Ca toxicity symptoms were observed during the head initiation stage in plots that received rock phosphate. Highest and lowest chlorophyll content with the levels of Limestone applied at 150% liming potential and control, respectively, where the highest mean value is 78.50 SPAD 502 value at 60 DAT where the chlorophyll content decreases after that to 65.70 to the same treatment at 90 DAT. The chlorophyll content of cabbage is highly responsive to calcium fertilizers, as reported earlier (Jamil *et al.,* 2007; Kang *et al.,* 2021). The highest chlorophyll content in the lime applied plot in the acidic soil was mainly due to Ca in exchange sites favouring the enhanced absorption of nutrients from the soil. Ca inside the plant system plays an influential role in converting absorbed nutrients into pigments (Dayod *et al.,* 2010).

Effect of treatments on ascorbic acid, titrable acidity, and peroxidase content

The ascorbic acid content in the cabbage is useful in scavenging the reactive oxygen species that induce oxidative stress and promote leakage of cellular electrolytes (Avalhaes *et al.,* 2009). Ascorbic acid increases with increasing Ca accumulation in cabbage. The highest ascorbic acid content was registered in the treatment that received Limestone and dolomite with the mean value of 76.4 mg $100g^{-1}$ (p=0.05) compared with other sources; this is in line with the findings of da Silva *et al.* (2021). There are few reports that ascorbic acid content increases with increasing Ca increase absorption on brassica species such as broccoli and cauliflower but not on cabbage (Barreto *et al.,* 2017; De Souza *et al.,* 2019). The highest titrable acidity was observed in cabbage with the mean of 0.61% from the treatments and levels of dolomite with 150% liming potential and $CaC0_3$ with 150% liming potential and was on par with each other. The increase in ascorbic acid and titrable acidity improves the firmness of the cabbage head, which in turn increases the shelf life. A sufficient amount of Ca provides a firmer head, improving the market value. The Peroxidase enzyme belongs to the group of oxidatoreductive enzymes that catalyses the scavenging reaction of Reactive oxidative species (ROS), a partially reduced form of atmospheric oxygen that damages the plant cell (Slesak *et al.,* 2017). The highest peroxidase was recorded due to the application of limestone $_{125\%LP}$ with an average of 0.45 unit's min^{-1} mg^{-1}. Hybrid Saint produces more peroxidase (0.62 units min^{-1} mg^{-1}) in the limestone application, implying

Table 2. Average calcium content and oxalate content on the edible equatorial region of cabbage head treated in four calcium sources and levels. Letter a, b, c, d, e, f, g, and h denote a significant difference in Calcium among the treatments at statistical analysis using ANOVA table p≤0.05

Ca Sources	Ca content in cabbage head mg $100g^{-1}$															
	$CaCO_3$				Rock phosphate				Dolomite				Ca-EDTA			
Levels Hybrids	100%LP	125% LP	150% LP	175 %LP	100% LP	125% LP	150% LP	175% LP	100% LP	125% LP	150% LP	175% LP	100% LP	125% LP	150% LP	175%LP
Tekila	38.3^{f}	45.6^{cd}	53.2^{b}	57.1^{a}	44.5^{d}	46.7^{cd}	47.1^{c}	45.3^{cd}	31.2^{g}	43.3^{de}	47.9^{c}	56.7^{a}	39.9^{f}	41.2^{ef}	44.6^{cd}	45.6^{cd}
Quisto	31.1^{g}	39.3^{f}	56.1^{ab}	57.8^{a}	39.9^{f}	51.2^{cd}	43.8^{e}	53.2^{cd}	43.2^{e}	49.8^{d}	51.2^{cd}	51.5^{cd}	32.8^{g}	33.4^{g}	37.8^{f}	40.1f
Saint	37.2^{g}	41.2^{ef}	49.9^{b}	55.5^{a}	41.2^{ef}	41.2^{de}	47.8^{bc}	47.9^{bc}	43.2^{de}	45.6^{cd}	49.8^{b}	51.2^{b}	38.9^{fg}	39.1^{fg}	43.2^{de}	43.9^{de}
NBH Nova 50	39.9^{e}	59.9^{a}	56.7^{b}	61.3^{a}	39.7^{e}	45.6^{d}	49.9^{c}	54.6^{b}	44.3^{d}	46.7^{cd}	46.9^{cd}	49.8^{c}	33.4^{f}	36.7^{ef}	39.9^{e}	35.6^{f}

Ca Sources	Oxalate content (mg 100 g^{-1})															
	$CaCO_3$				Rock phosphate				Dolomite				Ca-EDTA			
Levels Hybrids	100% LP	125% LP	150%LP	175% LP	100% LP	125%LP	150% LP	175% LP	100% LP	125% LP	150% LP	175% LP	100% LP	125%LP	150% LP	175% LP
Tekila	0.31^{b}	0.16^{de}	0.14^{ef}	0.10^{fg}	0.44^{a}	0.12^{fg}	0.10^{g}	0.10^{g}	0.19^{d}	0.11^{fg}	0.11^{fg}	0.13^{efg}	0.18^{d}	0.23^{c}	0.19^{d}	0.11^{fg}
Quisto	0.22^{ab}	0.19^{bc}	0.18^{bc}	0.17^{cd}	0.14^{e}	0.11^{e}	0.11^{e}	0.10^{e}	0.18^{bc}	0.14^{de}	0.13^{e}	0.12^{e}	0.24^{a}	0.21^{ab}	0.21^{abc}	0.19^{bc}
Saint	0.24^{a}	0.18^{bcd}	0.21^{abc}	0.17^{cd}	0.13^{ef}	0.13^{ef}	0.13^{ef}	0.12f	0.18^{bcd}	0.15^{def}	0.16^{de}	0.13^{ef}	0.18^{bcd}	0.21^{abc}	0.22^{ab}	0.21^{ab}
NBH Nova 50	0.32^{a}	0.24^{bcd}	0.20^{ebcde}	0.22^{cdef}	0.17^{gh}	0.19^{efgh}	0.17^{gh}	0.16^{h}	0.25^{b}	0.22^{defg}	0.24^{bcd}	0.25^{bc}	0.22^{bcdc}	0.19^{efgh}	0.18^{fgh}	0.17^{gh}

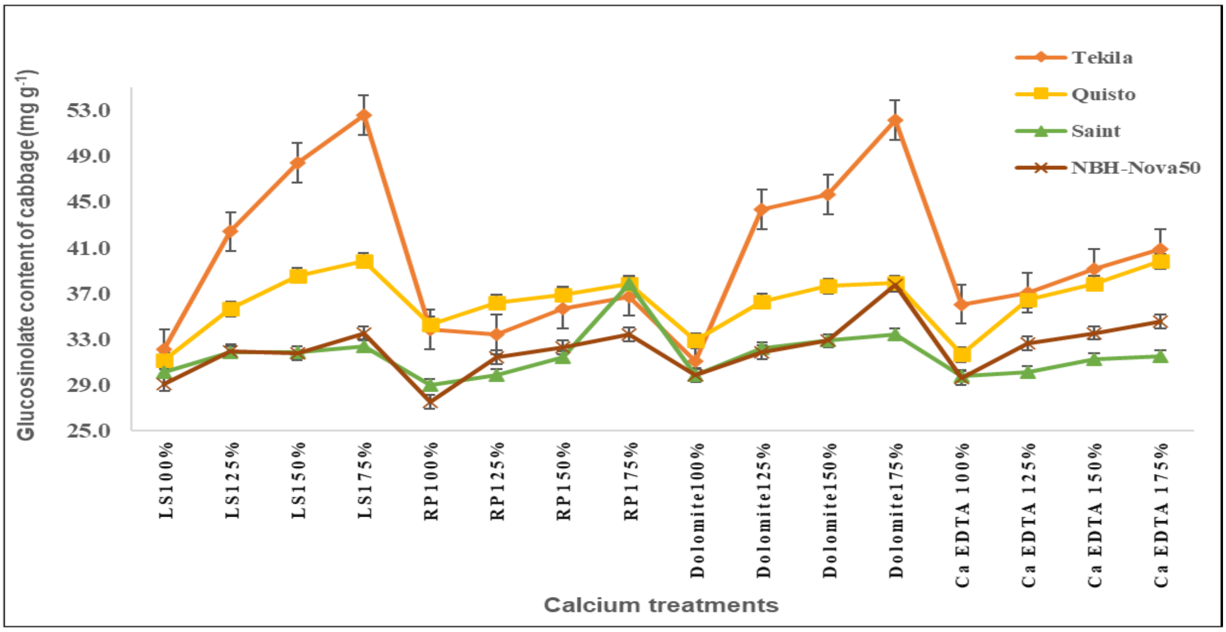
high antioxidant enzyme activity. In untreated calcium plots, low stomatal conductance causes high waterless through stomata that increase oxidative stress in plants which are common in acidic soil conditions reported in similar findings (Ma *et al.,* 2020).

Effect of treatments on plant oxalate and glucosinolates content of cabbage

Cabbage is one of the low oxalate crops, a suitable target for the calcium biofortification process as it does not get complexed to form calcium oxalate. The oxalate content shows significant variations among the treatments where a high oxalate found in the plot was treated with $CaCO_3$, both at 100% liming potential and 175% in the hybrid Quisto with the mean values of 0.32 mg 100 g^{-1} and 0.24 mg $100g^{-1}$ ($P<0.05$) respectively. Our quantification of oxalate content in cabbage is similar to the findings of previous studies (Camara- Martos *et al.,* 2021; Chang *et al.*, 2022; Managa *et al.,*2020). Some agronomic practices are proven to reduce the oxalic acid content of the crops, such as applying lime and nitrogenous fertilizers. So liming in cabbage reduces oxalic acid accumulation in the cabbage tissues. Typically Brassica family is oxalate free, making them a suitable target for essential mineral fortification. However, high oxalate intake leads to secondary hyperoxaluria, the precursor for kidney stone formation.

Glucosinolates and their hydrolysed derivatives are the principal components of the Brassica family, having cancer-protective effects (Rungapamestry *et al.,* 2006).The highest amount of glucosinolates concentration found in the plot treated with Dolomitic Limestone (150%LP) with an average mean value of 40.48 µmol g^{-1} dry weight, which is on par with the $CaCO_3$ (150% LP), which is 13.2 times higher amount compared to unbiofortified plants. The inner section of cabbage contains more glucosinolates than the outer leaves in all treated plants in all experimental plots, similar to (Choi *et al.,* 2014). The cabbage received Ca-EDTA and Rock phosphate, which were not significantly different from the control plot. Similar results were obtained in Chinese cabbage treated with lime produces more glucosinolates (Kim *et al.,* 2015; Lee *et al.,* 2019).

Regarding the hybrids, the Saint contains more glucosinolates concentration, followed by NBH-Nova 50, Saint, and Tekila. All four hybrids are green cabbage cultivars where glucosinolates are synthesized from homo-methionine, which belongs to the Nitrogen and sulphur-containing compounds (Rosen *et al.,* 2005). The increase in glucosinolates activity is directly proportional to the increased concentration of bioactive compounds like phenols and vitamin C in the brassica family, which mainly counteracts stress conditions (Camara-Martos *et al.,* 2022).



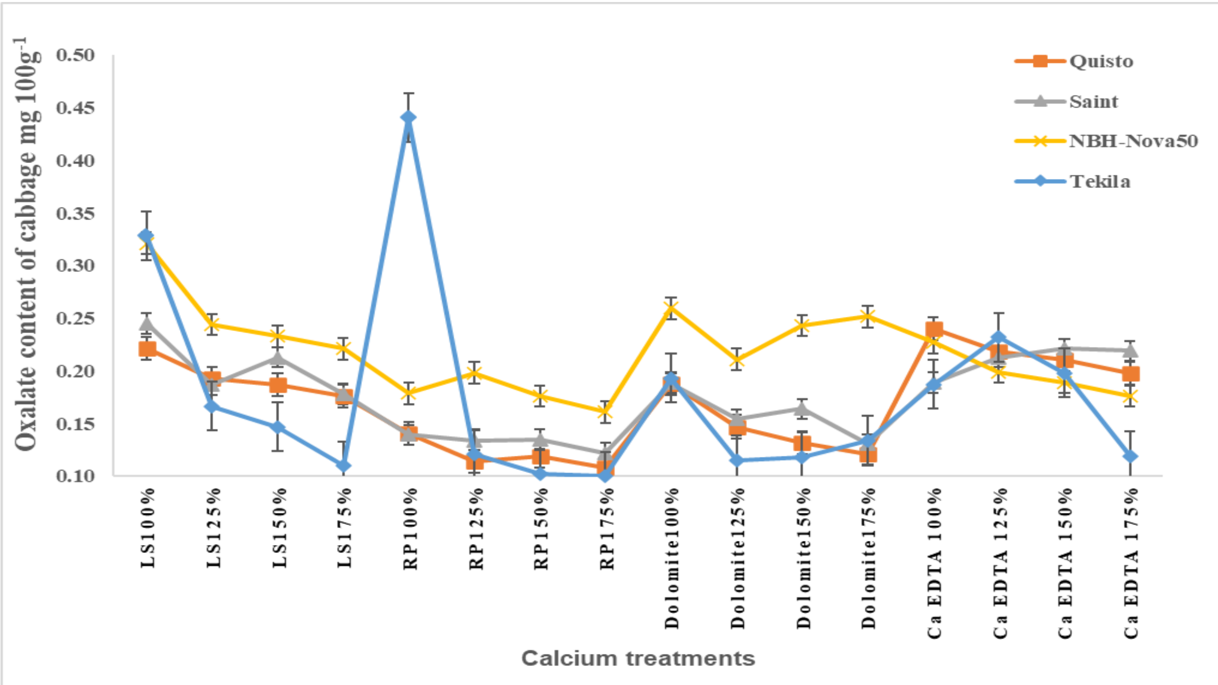


Fig. 1. *Glucosinolates and oxalate content of four cabbage hybrids about different Ca sources and levels of liming*

Effect of treatments on calcium content

The kinetics of calcium accumulation in the head of cabbage was assessed at the harvest stage. The head and foliage parts analysed for calcium content to estimate source-related Ca accumulation showed the highest Ca in the treatment of Limestone$_{175\%LP}$ at hybrids Tekila and NBH Nova 50 with 61.0 mg 100 g^{-1} and 59.8 mg 100g^{-1}, respectively, on par with the Limestone$_{125\% LP}$ with Ca content of 57.7 mg 100 g^{-1}. Calcium is biofortified in the treated plots of $CaCO_3$ and $[CaMg(CO_3)_2]$ compared with cabbage grown in Ca-EDTA and Rock phosphate. . The tissue calcium in the cabbage head ranges from 45.6-60.7 mg 100g^{-1} DW in the treatment; a 20% increase compared to the control. This Ca-EDTA (90 mg 100g^{-1} FM) showed high Calcium biofortified in cabbage tissues received both 150 and 175 % lime applied according to the liming potential. The highest Ca gets accumulated in the Tekila (on average of 102 mg 100g^{-1} FM), followed by Saint, NBH Nova 50, and Quisto. After the 3rd application of fertilizers, Ca-EDTA treated plots showed microscopic symptoms of Phosphorus deficiency in the leaves.

Similarly, plots that receive rock phosphate show Ca cause toxicity symptoms such as venial chlorosis and discoloration of inedible parts that affect the marketability of the cabbage head. An increase in Calcium appli-

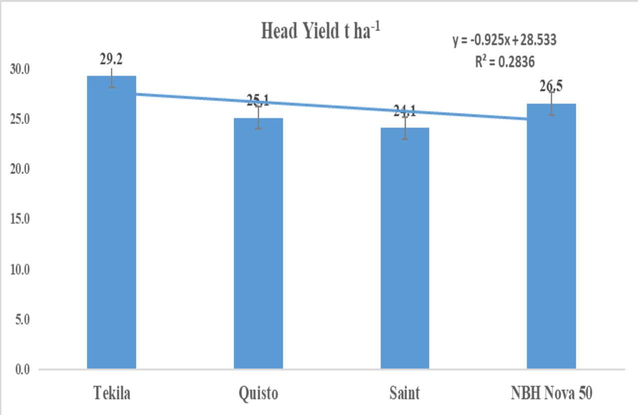


Fig. 2. *Head Yield of cabbage from four different hybrids applied with other Ca sources and levels of liming*

cation causes a significant increase in tissue Ca content in all biofortified plots compared with unbiofortified ones, with an enrichment percentage of about 12.3% on average. At the harvest stage, Ca biofortification in the whole head was comparatively higher in all treatments, with the average Ca biofortification index of about 51%-66%. Ca biofortified in the edible part were heterogeneously distributed within edible parts. The results of the present studies are consistent with the experimental findings of (Coelho *et al.,* 2021; Pessoa *et al.,* 2021; D'Imperio *et al.,* 2016). There are many experiments (Borghesi *et al.,* 2013; Colonna *et al.,* 2016) that biofortified Calcium in a soilless hydroponic system where the calcium content is attributed to different growth systems. Similar biofortification studies using

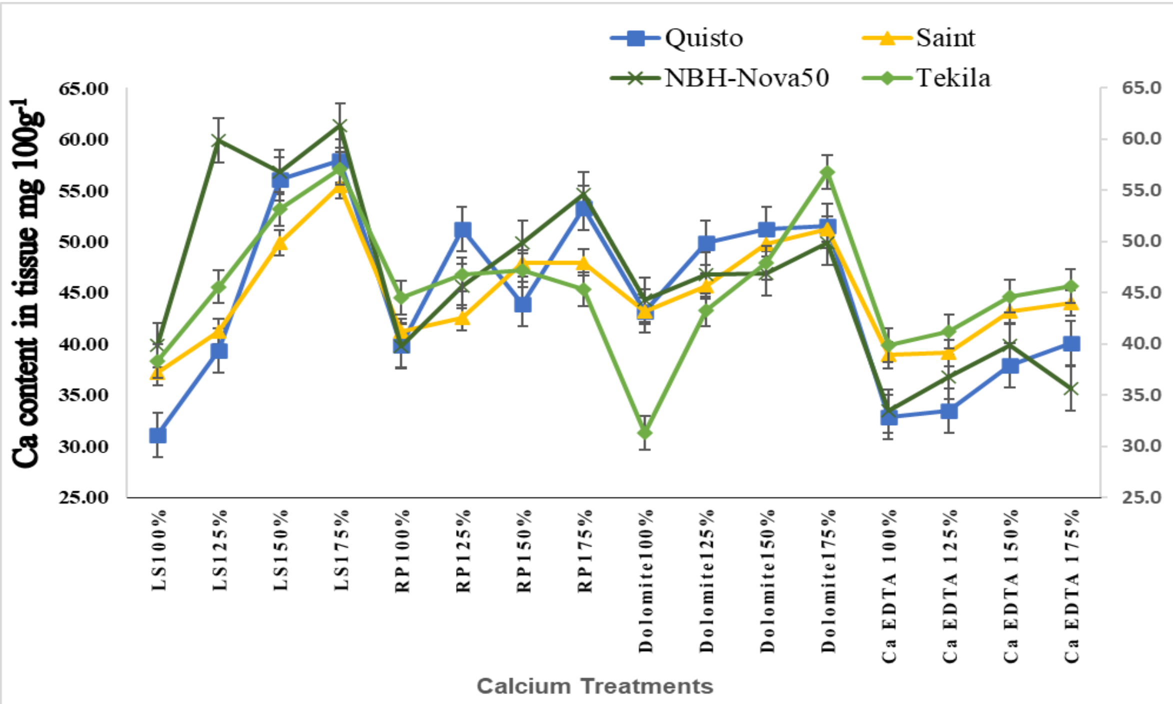


Fig. 3. *Calcium content in the edible portion of cabbage from four different hybrids applied with different Ca sources and levels of liming*

Rocha pears (Pessoa *et al.,* 2021) concluded that orchards applied with $CaCl_2$ and $CaNO_3$ obtained a biofortification ratio of 63% more than trees that did not receive any treatments. Coelho *et al.* (2021) studied potatoes and found that tuber Ca content is higher in $CaCl_2$ treatment than in potato tubers that received Ca-EDTA. The increase in calcium content in our research is in line with the findings of other authors that used agronomic practices, such as (Coelho *et al.*, 2021; D'Imperio *et al.,* 2016; Neeser *et al.,* 2005). The higher Ca content in the cabbage promotes more marketable quality and increases vegetables' shelf life (Yougen *et al.,* 2005). Regarding Calcium uptake, a high amount of Calcium is biofortified in the plot of Limestone and Dolomite at all levels compared with other treatments.

Effect of treatments on other inorganic ion content

The highest Mg content is observed in dolomite-treated plots (26.7 mg 100 g^{-1}), while magnesium content was observed to be lowest in Limestone treated plots, followed by rock phosphate. Other authors reported similar results (Burstrom (1968); D'Imperio *et al*., 2016; Koudela and Petrikova (2007); Upadhaya *et al*., 2017) in basil treated with different levels of Ca grown in hydroponic conditions. The calcium applied a Liming potential of 175% revealed a significant reduction in tissue magnesium content in all Ca biofortified compared to Calcium untreated. A linear decrease in tissue Mg content of about 16% was observed from the control plots. This reduction in magnesium was correlated with Ca content in cabbage (r^2=-0.35; p<0.001) in the hybrid

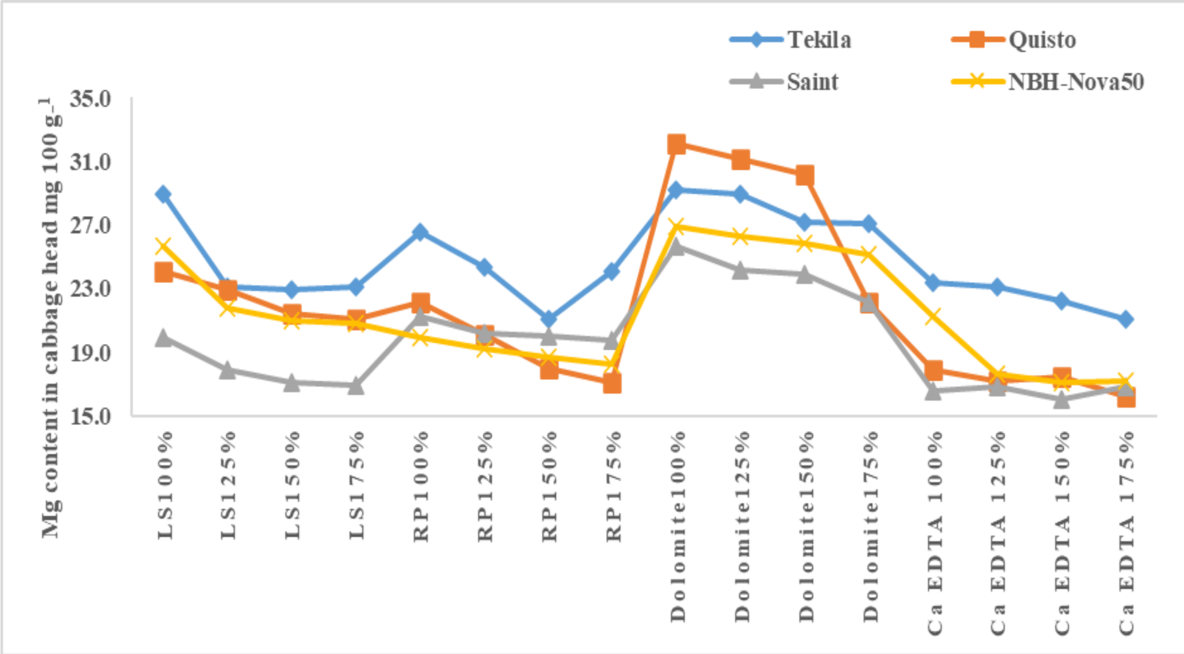


Fig. 4. *Magnesium content in the edible portion of cabbage from four different hybrids applied with different Ca sources and levels of liming*

Tekila which is followed by Quisto, Saint, and NBH Nova 50. The results conclude that antagonism between Ca and Mg was previously reported by Borghesi *et al.* (2013) and Tang and Luan (2017). Ca also have an antagonistic effect on Fe uptake where low Fe content is observed in the plot treated with all Ca fertilizers which significantly differ from each other. However, Ca-EDTA treated showed high Fe uptake because EDTA readily complexed with metal cations that automatically show a high amount of Fe and Mg content in Ca-EDTA treated plots with other Ca fertilizers. The highest Na content and K content in the plants were not significantly influenced by Ca fertilizers as they showed no significant difference from each other. Since potassium became the essential macronutrient for plant growth and development, it shows about 10% of dry weight in all hybrids, irrespective of the treatment applied. The Na and K content report are the same as the study conducted on Ca application in cabbage (Shin, 2014; Xie *et al*., 2021). Statically these Na and K content did not influence by Ca fertilizers in all hybrids while correlated. Plant nitrogen content in leafy vegetables is an essential component in marketable quality that affect colour and palatability. However, this Ca biofortification process did not increase the nitrate content in the cabbage, where all the total N content present was within the limit of 2.1-3.2 mg kg^{-1} FM, which was previously reported in lettuce (Santamaria, 2006) and cabbage (Atanasova, 2008; Zoo *et al*., 2014). Since high nitrogen content poses a serious threat to human health, it is important to maintain N content within standard limits in the biofortification process.

Conclusion

Cabbage grown in the acidic soil with the application of different sources and levels of calcium fertilizers did not show any visible symptoms of Ca toxicity at initial growth stages and did not increase the plant oxalate contents with the application of $CaCO_3$ at 150 % of liming potential compared to with dolomite, rock phosphate and Ca-EDTA fertilizers. Application of $CaCO_3$ (150% Liming potential) and $CaMg(CO_3)_2$ (175 % liming potential) as soil application promoted biofortification of cabbage irrespective of the type of hybrids cultivated in open field conditions. Besides, Ca-EDTA and rock phosphate did not significantly increase any plant calcium content. Specifically, this experiment indicates that consumption of 100g of biofortified cabbage is enough to meet the recommended dietary allowance of Calcium. This calcium biofortification process did not substantially affect the physicochemical characters except for the head firmness, which makes an additional customer satisfaction guarantee in marketability. Calcium fertilization at higher levels under high humid conditions leads to the accumulation of Ca-oxalate crystals in plant cells; that criteria make it mandatory to choose the crop or species with low oxalate content for the biofortification process that guarantees the Ca bioavailability to humans.

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Conflict of interest
The authors declare that they have no conflict of interest.

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