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EFFECTS OF CALCIUM-CONTAINING NATURAL FERTILIZER ON *CAMELLIA SINENSIS* (L.) KUNTZE

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Abstract

The effects of application of calcium-containing natural fertilizer on the functional state of the tea plant were studied. Application of calcium resulted weakening of the negative effects of high temperatures and water deficiency due to an increase in its heat resistance (on average by 30 - 40%), an increase in catalase activity (on average 5 - 10 %), as well as adaptive rearrangement of pigment ratio to increasing the content of carotenoids, chlorophylls and the their functional activity. In general, the more efficient functioning of the signaling intracellular network due to the calcium application provided better adaptability of plants to extreme conditions and more efficient recovery after subsequent rehydration, which in a whole contributed to an increase in shoot growth activity (on average 20%) and an increase in yield by an average of 27 - 33%.

The Black Sea coast of North-Western Caucasus is the one of the northernmost region where the tea plant is commercially cultivated. This crop was introduced in the region for the first time in 1878. The first tea plantation was founded here in 1901 and commercial propagation has been continuing till now. Tea in Russia, as well as in a several other regions of the world (China, India, Japan) faces such environmental factors as temperature stresses, solar insolation, drought, mineral deficiency which decrease its yield and productivity (Bhagat *et al.* 2010, Baruahl and Bhagat 2012, Malyukova 2014, Samarina *et al.* 2017). In this regard, it is important to study the effectiveness of various exogenous inductors (mineral fertilizers, biologically active compounds) to increase resistance and productivity of plants under extreme environmental conditions (Song *et al*. 2008, Njoloma 2012, Upadhyaya *et al*. 2012 and Pan Zhu-Cai 2015).

Among a wide range of biogenic macro- and microelements entering agro-ecosystems with fertilizers, the calcium is of special scientific interest which performs a signal function in the plant organism in the synthesis of stress proteins that provide resistance to unfavorable environmental factors, as well as recovery after stress (Bush 1995, Gao *at al.* 1999, Shu and Fan 2000, Li *et al.* 2002, Saidi *et al.* 2009). It was reported that Ca^{2+} is involved in regulating such diverse and fundamental processes as cytoplasmic streaming, thigmotropism, gravitropism, cell division, cell elongation, cell differentiation, cell polarity, photomorphogensis, and plant defense and stress responses (Reddy 2000, Malho *at al*. 2006). It is believed that calcium influx and cytoplasmic calcium ($[Ca^{2+}]$cyt) are important for guard cell ABA transduction, while ABA can regulate stomatal aperture in guard cells in many stress responses. In *Arabidopsis thaliana* the oscillating amplitudes of $[Ca^{2+}]$o (extracellular Ca^{2+} concentration) and $[Ca^{2+}]$i (cytosolic Ca^{2+} concentration) are controlled by soil Ca^{2+} concentrations and transpiration rates. Enhanced tolerance to temperature stress can also be explained by increased activity of γ- glutamyl kinase and decreased activity of proline oxidase in plant tissues (Hetherington and Brownlee 2004, Kim 2009). A number of studies have shown the effect of calcium on the reduction of oxidative damage in various plants (including tea) under drought by inducing an antioxidant system (Upadhyaya *et al.* 2012, Li *et al.*

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2004, Rikhvanov *et al.* 2014, Medvedev 2015). With prolonged cultivation of tea on naturally acidic soils, soil acidification is usually developed, leading to a significant decrease of available forms of calcium and the formation of a deficiency of this element (Malyukova 2014). Based on the available reports, the goal of the present work was to study the influence of the root application of calcium in the natural form on the functional state of tea and its nutritional status in field conditions with insufficient water supply. Application of calcium containing fertilizers and natural calcium-containing components has environmental and nature restoration effects and will help to improve the supply of plant with this element.

Experiments were conducted in the humid subtropical conditions of the Black Sea coast of the North-Western Caucasus (Russia, Sochi city, UchDere district - 43,69◦N., 39,64◦E.), wherein the average temperature varies from 12.8 - 16.5°C, annual rainfall of 1313 - 2098 mm. The growth period lasts from April to October, with the summer rainfall deficit. The observations were carried out during 2011 - 2017 in field plantations of mature tea plant *Camellia sinensis* commercial cultivar Kolkhida. The type of soils of the experimental plot is Cambisols, characterized by a high level of fertility and a sufficient number of nutrients for tea plant. The following treatments were laid out in a RBD: T1 - control, application $N_{240}P_{70}K_{90}$ in kg/ha; T2 - application $N_{240}P_{70}K_{90}$ in kg/ha along with a clay-chalk substance (CaO), which was applied at a dose of 100 kg/ha. There were three replications in each treatment. The area of each replication was 10 m^2. The plants were not irrigated as this is the general practice in this region. As a source of exogenous calcium, a natural material was used - a clay-chalk substance. The yields observations were made annually according to the experimental treatments during the periods of the formation of a 3-leaf young tea shoot. The total number of shoots was taken into account, as well as the ratio of different-aged shoots and their mass was carried out on the experimental field sites of 0.25 m^2 at 2 points on each experimental repeat. The content of tannins was determined in the aqueous extracts of tea leaves by volumetric permanganate method of Levintal, based on the oxidation of tannins with the action of 0.1N $KMnO_4$ in the presence of indigosulfuric acid, which acts both as an indicator and a regulator of the reaction. The content of extractive substances was determined by the weighting method according to Vorontsov (Voronzov 1946). The content of photosynthetic pigments (chlorophyll a and b, carotenoids) was determined in 100% acetone extract of fresh leaves using spectrophotometry at 665 nm wavelength for chlorophyll a, 649 nm for chlorophyll b and 440 nm for carotenoids using Ziegler and Egle computational formulas (Shlyk1971). Catalase activity in green leaves was determined gasometrically (Gunar 1972).

Assessment of the functional state of the photosynthetic apparatus of tea plants was carried out according to the parameters of slow induction of chlorophyll fluorescence on an LPT-3C device, according to instructions developed by Budagovskaya (Budagovskaya 2001). The efficiency of photochemical reactions was evaluated in terms of the relative fluorescence quenching-viability index (ratio of the maximum fluorescence to the stationary level of chlorophyll fluorescence. Heat resistance was evaluated in the dynamic using leaves according to Kushnerenko (Kushnerenko *et al.* 1985). The concentration of cell sap (CCS) was assessed by the refractometric method of Filippov (Filippov 1975). The count of microorganisms in the rhizosphere locus of soils (at a depth of 10 - 20 cm) was measured in the reconstituted samples after storage in a freezer at a –18°C by dilution and seeding of soil suspension onto elective media: MPA for bacteria, Gause-1 with the addition of penicillin (1.0 mg/l) and nystatin (50 mg/l) for actinobacteria and acidified Czapek medium for micromycetes (Zvyagintsev 1991). The processing of the experimental material was carried out using the methods of variation-descriptive statistics using the Agrochemistry program.

In favorable weather conditions, the yield of tea plant cultivar Kolkhida was on the average of 7200 - 9700 kg/ha (Table 1) and is comparable to the potential for this cultivar in the mentioned climatic conditions in the region (Malyukova 2014). The seasonal yield varies primarily as a result of seasonal temperature changes and soil drought stress during the dry season. The main part of the tea yield under these conditions was formed fairly evenly, mainly in the period May-early August (Table 2), with a bit advantage of the May harvest. In the unfavorable spring conditions, yield decreased in the May to 22 - 32% of all harvest. The maximum loss of the total yield (up to 50 - 60%) was observed in the drought period of July - August and was associated with the inhibition of plants by increasing Cell Sap concentration to 11.8 - 13.8% (when the optimum was 8 - 9%) and decreasing in shoot-growth ability (Tuov *et al.* 1997, Malyukova *et al.* 2016).

Table 1. Total yield of Kolkhida tea cultivar in various meteorological conditions.

Variant	Yield average (kg/ha)			Increase of yield (%)		
	1	2	3	1	2	3
Control	5400 ± 2700	7200 ± 1700	2800 ± 200	-	-	-
Ca treatment	6500 + 3700	8800 ± 2700	3100 ± 1300	20	22	11

1 - The whole period of 2011-2015, 2- years with favorable weather conditions (2011, 2013, 2014) and 3 - years with unfavorable weather conditions (2012, 2015).

The positive effect of using calcium fertilizer was observed for several years (Fig. 1), and most clearly it was observed in the second wave of tea growth (in different years, June, July, August) especially under unfavorable weather conditions such as hyperthermia and water deficiency (Table 2).

Table 2. Dynamics of tea yields during three-year growth period, centner/ha.

Month	2011*		2013*		2015**	
	1	2	1	2	1	2
May	23.1	20.6	26.4	31.0	4.7	9.4
June	4.5	6.7	22.0	26.2	10.4	16.0
July	17.9	19.4	18.0	26.9	4.2	6.5
August	7.1	9.9	20.1	25.8	9.6	8.5
September	5.9	4.2	5.0	6.7	0.0	0.0
Total	58.6	63.7	91.5	116.6	29.0	40.4
	s	t	s	t	s	t
LDS ($p < 0.05$)	2.1	1,9	3,3	2.5	1.0	3.4

*Years with favorable conditions, **With unfavorable conditions, 1 - Control (background), 2 -background + calcium. S - Season; t - Treatment.

In 2013 it was noted that after the dry season of 2012, tea plants with calcium-fertilizer treatment were recovered more quickly with an average yield increase of 5 - 8 centner/ha in comparison with the control. In unfavorable weather conditions of 2015 with a long period of drought (June - August) the maxima yield was observed on the variant with the calcium application.The increase in yields in the variant with calcium application was due to a better shoot-formation (Table 3), while the mass of young 3-leaf shoots was commensurable.

According to the content of tannin and extractive substances (averaged 3-year data) there was a tendency to increase the synthesis of the substances in the variant with calcium application

(Table 4). In general, by July, when drought stress usually limits the growth rates of shoots, an increase in the tannins content (to a lesser extent, extractive substances) was observed comparing with the May period, which is a specific character of tea growth in this region.

Table 3. Total amount (number/m^2) and mass (g) different-aged tea flushes for full growth period.

Variant	Mean number of different-aged shoots obtained from 8 harvesting					Total mean amount of shoot for all harvest period
	Bud	2-leaf flush	3-leaf flush	4-leaf flush	Full number	
Control	202 ± 31* 0,63 ± 0,07**	418 ± 25 0,57 ± 0,05	375 ± 119 0,98 ± 0,11	25 ± 15 1,66 ± 0,13	998 ± 203	1297
Ca	271 ± 42 0,69 ± 0,12	446 ± 115 0,58 ± 0,04	467 ± 83 0,95 ± 0,07	34 ± 11 1,59 ± 0,21	1194 ± 245	1552

*The numerator is the total number of shoots, **The denominator is the mass of the shoot parameters quality tea.

Table 4. The content of tannin and extractive substances in the young 3-leaf shoots of tea plant cultivar Kolkhida (average for 2011-2013).

Variant	Tannin (%)		Extractive substances (%)	
	May	July	May	July
Control	25.3 ± 1.9	31.3 ± 1.0	41.3 ± 0.9	43.1 ± 1.0
Ca	27.2 ± 2.1	32.8 ± 1.3	41.9± 0.8	43.8 ± 2.0

The content of photosynthetic pigments in the mature leaf and young 3-leaf shoots varied according to the experimental treatments, the higher content of chlorophyll a over chlorophyll b was observed (Table 5). In the treatment of calcium application, the absolute content of all the pigments was lower in comparison with the control, but an increase in the carotenoids content per unit of chlorophylls was observed, which indicated a more effective rearrangement of the photosynthetic pigments reserve under stressful conditions.The changes observed in the structure of the photosynthetic apparatus were referred to its functional activity. So, in the variant with calcium application, there was a significant increase (August 2012), or a growth trend (August 2014) of the viability index in comparison with the control (Fig. 1).

The heat tolerance of the leaves during the increasing stress in the variant with calcium application exceeded the control, which was revealed by a reliable decrease in the "T1-T2" and "T2 / T1" indicators (Table 6). The effect of factor-fertilizer for these indicators was 61 - 68%.

The activity of the catalase antioxidant enzyme in mature leaves of the tea plant under stress conditions (Fig. 2) was much higher than in favorable conditions (Belous 2006), which is the primary signal indicating the occurrence of oxidative stress, as long as catalase, along with other compounds, plays a key role in regulating active oxygen species level (Bowler and Fluhr 2000, Li *et al.* 2004, Medvedev 2005).

The activity of the enzyme in mature leaves was higher using the calcium-containing fertilizer, in all studied periods (except August 27, 2015), indicating the evidence of oxidative damage recovery during drought by inducing the plant's antioxidant system, as noted by other researchers (Upadhyaya *et al.* 2013).

Table 5. The content of photosynthetic pigments in the mature tea leaf *cv.* Kolkhida in summer (2011-2013).

Variant	Chlorophyll			Carotenoids	Ratio	
	a	b	a + b		a / b	a + b / k
	mg/g fresh weight					
25. 07. 2011						
Control	1.43 ± 0.05	0.99 ± 0.08	2.42 ± 0.13	0.60 ± 0.04	1.44 ± 0.06	4.03 ± 0.09
Ca	1.33 ± 0.18	0.90 ± 0.21	2.23 ± 0.40	0.56 ± 0.10	1.48 ± 0.16	3.98 ± 0.08
23.08.2012						
Control	1.95 ± 0.09	1.29 ± 0.12	3.25 ± 0.20	0.65 ± 0.03	1.52 ± 0.07	4.99 ± 0.16
Ca	1.78 ± 0.11	1.07 ± 0.14	2.85 ± 0.25	0.67 ± 0.06	1.68 ± 0.12	4.28 ± 0.25
10.07.2013						
Control	1.88 ± 0.06	1.14 ± 0.16	3.02 ± 0.21	0.64 ± 0.07	1.67 ± 0.20	4.80 ± 0.10
Ca	1.48 ± 0.14	1.39 ± 0.22	2.83 ± 0.33	0.74 ± 0.08	1.02 ± 0.09	3.95 ± 0.12

In Tables and Figure, control is - $N_{240}P_{70}K_{90}$ and treatment is Ca - $N_{240}P_{70}K_{90}$+ Ca.

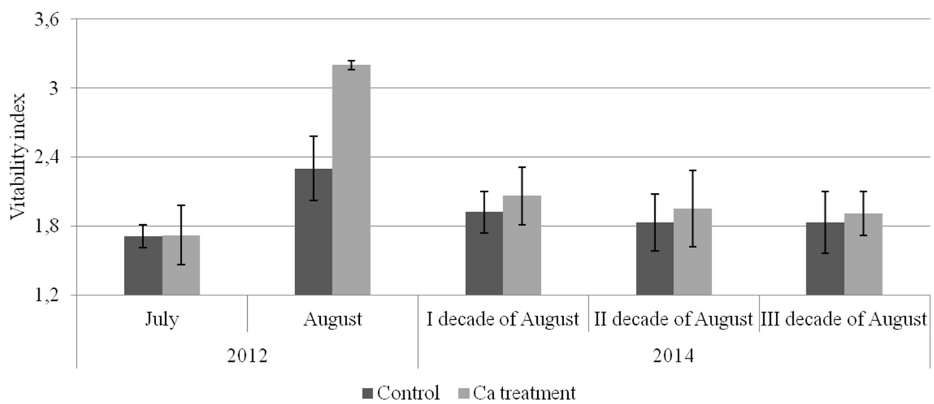

Fig. 1. Viability index measured in mature tea leaf during drought stress period.

When the calcium-containing fertilizers were applied into the soil, the structure of the soil-absorbing complexchanged in the direction of increasing calcium and magnesium content in it, along with the decrease of ammonium nitrogen and phosphate ions content in comparison with the control (Malyukova *et al.* 2016). In the calcium application treatment, the increase in the number of soil actinobacteria, micromycetes, and bacteria was less observed (rhizosphere coefficient 2 - 4) comparing with control (rhizosphere coefficient 0.3 - 1)in the tea rhizosphere area that is the evidence of a higher functional activity of the studied biocenosis (microbiocenosis + phyto-cenosis) (Fig. 3).

In general, plants suffer from repeated short or long high-temperature stress in conjunction with low moisture content. Early recognition upcoming stress effects was very important for the timely start of protective mechanisms, in particular the accumulation of molecular substances, regulate specific membrane channels, etc. According to the published data, the change in calcium concentration in the cytosol is the first step in cell recognition external impact and launch transduction system (transmission) signal (Shu and Fan 2008, Upadhyaya *et al.* 2011). It is also

Table 6. Indicators of leaf heat resistance.

Variant	Year	Indicators			
		T1-T2		T2/T1	
		04.08	18.08	04.08	18.08
Control	2015	5.93 ± 2.58	8.39 ± 1.74	0.68 ± 0.10	0.57 ± 0.09
	2016	8.02 ± 2.20	-	0.57 ± 0.08	-
Ca	2015	5.68 ± 1.59	4.75 ± 1.42	0.70 ± 0.09	0;76 ± 0.08
	2016	4.89 ± 2.39	-	0.72 ± 0.11	-
LSD_{005}		2.10	1.25	0.07	0.05

T1-T2 - The thickness of the leaf before and after heating, respectively, at a temperature of 40°C and the air humidity preventing the sample cooling.

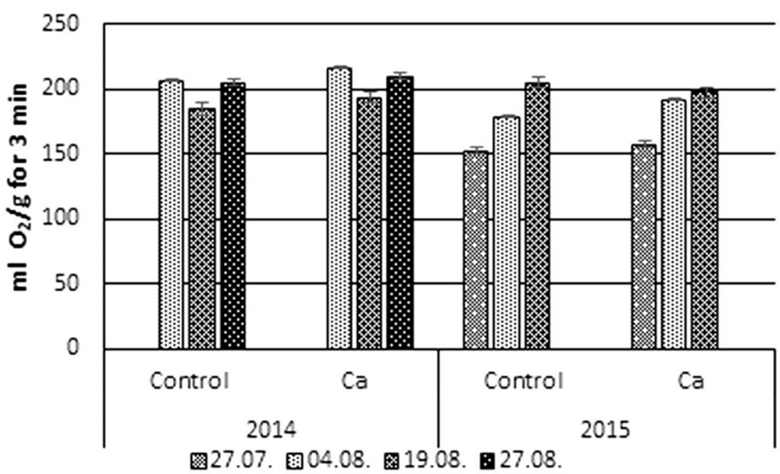

Fig. 2. Catalase activity (ml O_2/g for 3 min) of mature leaves of a tea plant in a water deficiency period.

known that calcium protects membrane thylakoids from damage (directly or indirectly) under stress conditions by increasing the activity of antioxidant enzymes and osmotic regulators contents and also participate in the reorientation of chloroplasts, their mechanical movement in order to more efficient absorption of light quanta (Bykhov 2004, Jarén-Galánand Minguez-Mosquera 1997, Fang *et al.* 2015). The use of calcium-containing fertilizers on acidic soils that compensate for the deficiency of exchangeable calcium has a positive effect by increasing its availability for plants, especially in dry years.

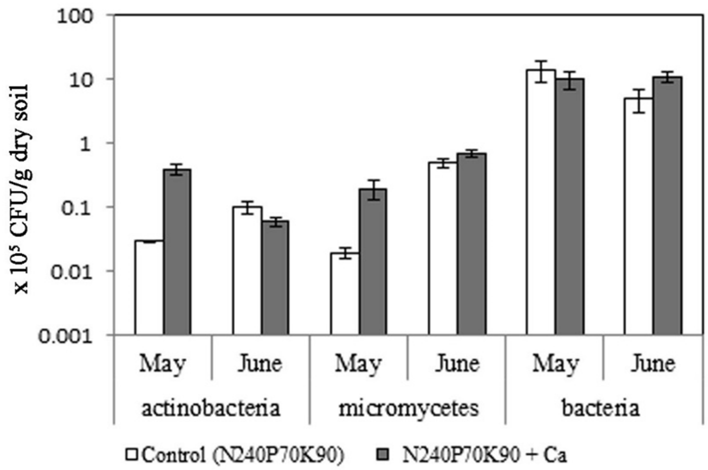

Fig. 3. Number of main groups of microbiocenosis of the rhizosphere locus of soils under the tea crop in 2015.

The use of calcium-containing fertilizer for growing tea led to a weakening negative effect of high temperatures and eliminate effect of water deficit on tea plant by increasing its heat resistance (an average of 30 - 40%), an increase in catalase activity in mature leaves (at 10 - 19 ml O_2/g for 3 min in different periods), and adaptive recovery of the pigment content. These processes showed more efficient operation of the intracellular signaling network undercalcium application, which are closely linked oxidizing and calcium signaling pathways (13 - 20), provided generally higher functional activity of plants under extreme conditions and during subsequent rehydration and substantial increase in yield (by 27 - 33%). More rearrangement adapted to extreme conditions plants and their subsequent efficient recovery occurred due to the effect of calcium on the cation exchange fertilizer composition soil-absorbing complex in the direction of increasing it exchangeable calcium content (40 - 60 mq/kg). These results will be useful for improvement of plant adaptation strategies to environmental stress factors.

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