Genotypic Variation in Calcium Uptake in Common Bean (*Phaseolus vulgaris* L.) under Chilling Stress

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Abstract: Plants are constantly exposed to different abiotic stresses throughout their lifecycle. Sub-optimal temperatures are important abiotic stress factors in agriculture. Plant growth, development, yield, and crop quality are significantly affected by low temperature stress. In the present study, 95 different common bean genotypes were grown in the growth chamber. As the control group, the growth chamber was designed to be 16/8 h light and dark with 25 °C and 20 °C day/night temperatures, respectively. Fifteen days old seedlings were exposed to chilling stress as cold stress treatment in the dark (night) at 4 °C for 8 hours and in light (day) at 25 °C for 16 hours. After fifteen days of low temperature stress, the experiment was completed to investigate calcium (Ca) intake in plants. Shoot Ca concentration of the genotypes were analyzed and genotypes were classified according to their Ca content. Therefore 24 genotypes were found to have less than ≤0.5%, 35 genotypes were found to have between 0.51 to1.0% and 36 genotypes were found to have between 1.01 to 3.0% Ca content under chilling stress.

Keywords: Cold stress, bean, calcium, abiotic stress, low temperature

1. Introduction

Climate change has been discusssed and evaluated since the late 1980s. Many aspects of the climate change has been evaluated by taking into account the internal and external forces and factors that may cause changes in the global climate system, as well as human activities that increase greenhouse gas accumulations (Türkeş, 2003).

Scientists have a great deal of consensus that climate is changing and will continue to be affected by future impacts. The adverse effects of these changes in the climate will affect the people who are already disadvantaged due to their region and living conditions. Especially low income farmers relying on agriculture and living in rural areas feel more destructive effects of climate change.

Stress is defined as the damaging power of various factors on plants. Stress causes changes in the growth and development of plants. Stress occurs at any time in the life of the plant and adversely affects the plants, causing the death of plants, organs or tissues.

For each plant to have normal growth and development, a certain optimum temperature range is required and temperatures below this are perceived as cold stress. Besides, certain temperature conditions may be optimal for a plant species and cause stress to the other (Mahajan and Tuteja, 2005).

Approximately 25% of the terrestrial area on the Earth is composed of regions that do not fall below 15 °C and are reliable for frost damage. Particularly cold-sensitive plants can be damaged due to the temperature falling below 0 °C in periods in the remaining areas (Sakai ve Larcher, 1987; Scebba et al., 1998; Zhao, 1998; Vagujfalvi et al., 1999; Pearce, 1999, 2001; Szalai et al., 2001; Puhakainen, 2004).

Signs of cold damage vary depending on the duration of exposure to cold on the plant

(genotype), the developmental stage, cold contact tissue, wind, water, nutrients and other environmental factors such as light (Saltveit and Morris, 1990).

In general, these symptoms cause reduced growth rate, and leaf width, while increase in cellular autolysis and aging, programmed cell death, chlorosis loss due to photo-oxidation, deterioration of cell membrane structures, consequent disruption of cellular integrity, reduced protoplasmatic flow and necrosis (Kratsch and Wise, 2000; Mahajan and Tuteja, 2005; Rymen et al., 2007).

Under low temperature stress, plants show a variety of cold-induced physiological and biochemical responses, such as the production of reactive oxygen species (ROS), changes in membrane lipid composition and changes in osmolytes (Thomashow, 1999; Browse and Xin, 2001). The mechanism of tolerance to chilling stress in plants is a complex network that is influenced by many factors. There is a need for in-depth evaluation of cold stress response mechanisms. Intra and intercellular frost formation mechanisms, frost formation in the tissues and dry matter content change, as well as lipids, proteins, some amino acids, antioxidant enzymes, macro, and micronutrients are thought to be important factors for the examination of changes in elements.

Calcium (Ca) acts as a secondary signal in plants against external impacts (Trewavas and Malhó, 1997). Various biotic and abiotic stimulants such as chilling, touch, light, pathogens, and plant hormones cause a temporary increase in cytosolic Ca concentration (Knight and Knight, 2000; Scrase-Field and Knight, 2003; Turan, 2007). The earliest response to low temperature was reported to be the temporary increase in free cytosolic Ca concentration (Monroy and Dhindsa, 1995; Turan, 2007).

Phaseolus sp. can undergo substantial hardening or acclimatization to low temperatures. Plant growth decreases in chilling stress. The activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase were increased in French bean under chilling stress, while β-carotene content was decreased (El-Saht, 1998).

The aim of the present study was to determine the effect of chilling stress on Ca content in common bean genotypes. Thus, bean genotypes were classified according to Ca content under chilling stress.

2. Materials and Methods

The experiment was conducted in a growth chamber of the Department of Horticulture, Faculty of Agriculture at Çukurova University, Adana, Turkey. In the experiment, seedlings of common bean were studied. 95 different bean (*Phaseolus vulgaris* L.) genotypes were grown in the growth chamber under control and chilling stressed conditions. The seeds of the bean genotypes were sown in 2-liter pots containing vermiculite. For each genotype, 2 pots and 4 plants were grown in each replication. The growth chamber for the control treatments were arranged to 25/20 °C for 16/8 hours in light and dark, respectively. The plants were grown in these conditions for 15 days. After 15th-day, plants were exposed to 25/4 °C for 16/8 hours of light and dark, respectively. The nutrient requirements of the plants were supplied by irrigating with the nutrient solution. The mineral content of the nutrient solution is shown in Table 1.

Table 1. Total element concentrations in the nutrient solution used in the experiment

Elements	Concentration (mg L^{-1})
Nitrogen	130
Phosphorus	35
Potassium	220
Magnesium	45
Calcium	150
Sulfur	70
Iron	1.5
Manganese	0.8
Boron	0.5
Zinc	0.15
Copper	0.10
Molybdenum	0.10

The shoot of the plants was washed once with tap water and twice using deionized water. They were then dried in a forced-air oven at 65 °C for 48 hours and were ground (40 mesh sieve) for elemental analysis. Ground samples were dry shed in a muffle furnace at 550 °C for 6 h. The ash was then dissolved in 0.1 M HCl (hydrochloric acid) solution. Calcium concentrations were determined using an atomic absorption spectrophotometer (Jones, 2001).

The experiment was designed based on completely randomized experimental design. All data presented for Ca content were the average values of four replications of each treatment. Data were analyzed using JMP 7 software (SAS Institute Inc.). Independent samples t-test were used for comparison of plant Ca content under control and chilling stressed conditions. The data were also subjected to percent changes under chilling stress relative to control.

3. Results and Discussion

It was shown that chilling stress affected shoot Ca content of the common bean genotypes evaluated in this study. While Ca content of some genotypes was increased, some of them were stable or decreased.

The Ca concentration under chilling stress relative to control were higher in Bn-70, Bn-75B, Bn-153, Bn-103, Bn-72, Bn-76, Bn-6, Bn-62, Bn-114, Bn-30, and Bn-127, respectively (Table 2). However, Ca concentration under chilling stress relative to control were lower in Bn-106, Bn-131, Bn-74, Bn-57, Bn-63, Bn-15, Bn-16, Bn-29, Bn-32, Bn-24, Bn-112, and Bn-27, respectively.

When control and chilling stressed treatments were compared in terms of shoot Ca content by t-test, differences of twenty-six genotypes were not statistically significant. Ca concentration increases of chilled bean genotypes relative to control was observed between 2.11 to 478.13%. Ca concentration reduction on chilling stressed bean genotypes relative to control was between 1.69 and 96.1% (Table 2).

Under chilling stress, the best Ca contents were found in Bn-114, Bn-13, Bn-37, Bn-163A, and Bn-35, respectively. However, the lowest Ca contents were found in Bn-27, Bn-99, Bn-151, Bn-2, and Bn-15, respectively (Table 2).

Table 2. Shoot Ca concentration of the bean genotypes in control and chilling stress

Genotypes	Control (%)	Chilled (%)	t-test	Change in Ca concentration relative to control
Bn-2	0.21±0.02	0.31±0.01	0.0072*	47.62
Bn-4	0.62±0.10	0.35±0.02	0.0507^{ns}	-43.55
Bn-6	0.43±0.03	1.02±0.02	<.0001*	137.21
Bn-8	2.93±0.41	1.26±0.00	0.0002*	-57.00
Bn-9	0.60±0.22	0.72±0.01	0.4228^{ns}	20.00
Bn-10	1.48±0.21	2.01±0.01	0.0021*	35.81
Bn-12	1.72±0.37	1.60±0.11	0.7223^{ns}	-6.98
Bn-13	1.66±0.16	2.50±0.04	0.0190*	50.60
Bn-14	1.47±0.01	1.28±0.06	0.3318^{ns}	-12.93
Bn-15	1.16±0.01	0.32±0.02	<.0001*	-72.41
Bn-16	2.03±0.00	0.68±0.07	<.0001*	-66.50
Bn-17	0.76±0.04	0.46±0.05	<.0004*	-39.47
Bn-18	1.18±0.00	1.65±0.12	0.0054*	39.83
Bn-19	1.53±0.07	1.69±0.02	0.0790^{ns}	10.46
Bn-20	2.16±0.08	1.57±0.23	0.0002*	-27.31
Bn-22	2.24±0.09	1.68±0.11	<.0001*	-25.00
Bn-23	3.43±0.20	1.54±0.00	<.0001*	-55.10
Bn-24	2.27±0.03	0.83±0.16	<.0001*	-63.44
Bn-26	1.67±0.03	1.45±0.19	0.2827^{ns}	-13.17
Bn-27	0.71±0.03	0.28±0.00	<.0001*	-60.56
Bn-28	0.63±0.00	0.86±0.00	0.0157*	36.51
Bn-29	1.14 ±0.06	0.40±0.06	<.0001*	-64.91
Bn-30	0.69±0.06	1.36±0.09	0.0012*	97.10
Bn-32	1.05±0.03	0.37±0.03	<.0001*	-64.76
Bn-33	1.03±0.01	0.69±0.03	0.0080*	-33.01
Bn-34	0.89±0.11	0.52± 0.07	0.0051*	-41.57
Bn-35	2.17±0.29	2.10±0.07	0.8051^{ns}	-3.23
Bn-36	0.93±0.19	0.69±0.09	0.2870^{ns}	-25.81
Bn-37	1.33±0.03	2.25±0.17	0.0418*	69.17
Bn-38	1.53±0.22	1.78±0.00	0.3355^{ns}	16.34
Bn-39	1.80±0.20	0.66±0.13	0.0004*	-63.33
Bn-40	0.91±0.00	1.09±0.08	0.1402^{ns}	19.78
Bn-42	1.23±0.00	1.67±0.13	0.0005*	35.77
Bn-44	1.03±0.07	1.73±0.01	<.0001*	67.96
Bn-48	2.78±0.08	1.13±0.08	<.0001*	-59.35
Bn-49	1.59±0.12	1.39±0.10	0.1200^{ns}	-12.58
Bn-50	1.22±0.03	0.81±0.11	<.0001*	-33.61
Bn-51	1.27±0.03	1.07±0.07	0.2291^{ns}	-15.75
Bn-52A	1.18±0.14	1.16±0.00	0.8320^{ns}	-1.69
Bn-52B	1.50±0.07	1.39±0.13	0.2744^{ns}	-11.46
Bn-54	0.43±0.02	0.68±0.04	0.0013*	58.14
Bn-55	1.24±0.02	1.34±0.05	0.3833^{ns}	8.06

*: Significant, ns: non significant

Table 2. (continued)

Genotypes	Control (%)	Chilled (%)	t-test	Change in Ca concentration relative to control
Bn-56	0.96±0.03	0.43±0.18	<.0001*	-55.21
Bn-57	1.44±0.06	0.33±0.19	<.0001*	-77.08
Bn-60	1.43±0.14	1.73±0.23	0.0281*	20.98
Bn-61	1.08±0.11	1.26±0.00	0.0062*	16.67
Bn-62	0.74±0.01	1.58±0.13	0.0001*	113.51
Bn-63	0.64±0.06	0.16±0.02	0.0002*	-75.00
Bn-64	0.64±0.13	1.07± 0.18	0.0256*	67.19
Bn-65	0.66±0.05	0.41±0.03	0.0032*	-37.88
Bn-66	1.24±0.08	1.48±0.14	0.0119*	19.35
Bn-67	0.98±0.19	1.46±0.11	0.0089*	48.98
Bn-68	1.30±0.02	1.09±0.08	0.0534ns	-16.15
Bn-69	1.31±0.01	1.00±0.18	0.0466*	-23.66
Bn-70	0.32±0.14	1.85±0.34	<.0001*	478.13
Bn-71	1.09±0.12	1.20±0.04	0.5744ns	10.09
Bn-72	0.37±0.00	1.04±0.16	<.0001*	181.08
Bn-74	0.42±0.06	0.07±0.00	<.0001*	-83.33
Bn-75A	1.02±0.05	0.52±0.19	0.0080*	-49.02
Bn-75B	0.32±0.07	1.70±0.02	<.0001*	431.25
Bn-75C	1.31±0.01	0.66±0.10	0.0008*	-49.62
Bn-76	0.46±0.06	1.19±0.08	0.0034*	158.70
Bn-77	1.73±0.08	0.88±0.11	<.0001*	-49.13
Bn-79	0.45±0.09	0.33±0.04	0.0213*	-26.67
Bn-87	1.63±0.05	0.72±0.12	<.0001*	-55.83
Bn-89	0.59±0.04	0.37±0.05	0.0172*	-37.29
Bn-99	0.39±0.02	0.29±0.08	0.0255*	-25.64
Bn-101	2.81±0.01	1.93±0.15	<.0001*	-31.32
Bn-102	1.96±0.16	1.30±0.07	<.0001*	-33.67
Bn-103	0.10±0.00	0.40±0.16	<.0001*	300.00
Bn-104	1.26±0.18	1.19±0.02	0.3146ns	-5.56
Bn-105	0.65±0.07	0.43±0.06	0.0214*	-33.85
Bn-106	1.54±0.07	0.06±0.02	<.0001*	-96.10
Bn-111	1.18±0.04	1.22±0.08	0.6792ns	3.39
Bn-112	0.40±0.01	0.15±0.01	<.0001*	-62.50
Bn-114	1.81±0.25	3.76±0.33	<.0001*	107.73
Bn-116	2.68±0.00	1.64±0.05	<.0001*	-38.81
Bn-127	0.81±0.02	1.46±0.08	<.0001*	80.25
Bn-130	1.31±0.03	1.43±0.03	0.4573ns	9.16
Bn-131	0.49±0.00	0.07±0.16	<.0001*	-85.71
Bn-138	1.42±0.03	1.45±0.07	0.6100ns	2.11
Bn-139	3.21±0.00	1.41±0.08	<.0001*	-56.07
Bn-140	1.52±0.14	1.84±0.27	0.0822ns	21.05
Bn-147	0.82±0.08	0.62±0.05	<.0001*	-24.39
Bn-151	0.72±0.00	0.30±0.09	<.0001*	-58.33
Bn-152	0.31±0.05	0.33±0.12	0.4732ns	6.45
Bn-153	0.14 ±0.04	0.68±0.09	<.0001*	385.71
Bn-154	0.64±0.02	0.46±0.27	0.0004*	-28.13
Bn-157A	1.01±0.00	0.63±0.02	<.0001*	-37.62
Bn-157B	1.41 ±0.08	1.00±0.13	0.0678ns	-29.08
Bn-158	0.94 ±0.08	0.99±0.21	0.0278*	5.32
Bn-159	0.85±0.13	0.53±0.17	<.0001*	-37.65
Bn-162	1.69±0.16	1.06±0.02	0.0053*	-37.28
Bn-163A	1.71 ±0.01	2.13±0.00	0.5890ns	24.56
Bn-163B	1.02±0.07	0.53±0.11	0.1208ns	-48.04

*: Significant, ns: non significant

The Ca concentration of plants under control treatment ranged between 0.10% to 3.43%, while it was between 0.06% to 3.76 % in cold treated plants.

Cold acclimation is important and a universal phenomenon in chilling tolerant plants (Srivastava et al., 2015). Physiological and biochemical changes occur in plants during cold stress (Miura and Furumoto, 2013). The adequate Ca concentrations in bean leaves are between 0.50% and 2.00% under normal conditions (Favaro et al.,

2007). When plants are exposed to low temperature stress, Ca has been reported to be accumulated temporarily in the tissues of some alfalfa genotypes (*Medicago sativa*) (Aslantas et al., 2010; Puhakainen, 2004). In this study, chilling stress caused an increase in the Ca concentration on some genotypes. The least shoot Ca concentration was 0.06% (Bn106) and the highest was 3.76% (Bn114) under chilling stress in this study. Under chilling stress, it was reported that there was Ca flow from the apoplast to the cytoplasm fluid. The Ca channels in plasma membranes acts as sensors in a temperature drop. Ca increase in cells is one important phenomenon for the study of genes that react to low temperature stress (Aslantas et al., 2010; Smallwood and Bowles, 2002). Ca signaling plays a crucial role in conferring chilling tolerance in plants. Generally, plant genotypes that tolerate low temperature stress can maintain high leaf water potential by closing their stomata and preventing transpirational water loss (Wilkinson et al., 2001). Calcium is an essential requirement for chilling induced stomatal closure in chilling tolerant genotypes. It is also believed that ABA-induced stomatal closure is partially mediated by Ca released from internal guard cell stores or the apoplast (Wilkinson et al., 2001), and this function seems to make Ca a major contributing factor to chilling tolerance and protection of leaves from dehydration. It has been suggested that Ca is necessary for recovery from low temperature stress by activating the plasma membrane enzyme ATPase which is required to pump back the nutrients that were lost in cell damage (Palta, 2000).

It has been reported that Ca flow occurs from the apoplast to the cytoplasm fluid. Calcium channels in plasma membranes have been reported to act as sensors against temperature drop (Smallwood and Bowles, 2002). Additionally, frost resistance is higher in flower buds with high dry matter and Ca content (Aslantaş, 1999).

Plants have been reported to increase cytoplasmic Ca as an early response to low temperature stress (Leipner, 2009). Researchers have started to investigate the ways in which free cytosolic Ca^{2+} [(Ca^{2+})cyt] increase is triggered by low temperature stress (Minorsky, 1985) and oxidative stress occurs in low temperature-sensitive plants (Hariyadi and Parkin, 1993). In maize root cells, Ca^{+2} was reported to increase 20-25% at a temperature of 2 °C (Zocchi and Hanson, 1982). The cytosolic free Ca is a common secondary messenger in abiotic stress signaling. Threats to agriculture such as salinity, water scarcity, and hypoxia are signaled through calcium (Wilkins et al., 2016). Calcium influence on chilling resistance

of grafted eggplant seedlings were studied in a hydroponic system. Under the Ca sufficient treatment, the activities of peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) in seedlings at 5 °C were all significantly higher than those of Ca deficiency. Calcium content was potentially the main reason that grafted seedlings had higher chilling resistance than own-rooted seedlings. Calcium appears to play an important role in increasing the chilling resistance of grafting eggplant seedlings (Gao et al., 2005).

Bean genotypes were classified according to Ca concent responses under the chilling stress. According to this classification, twenty four bean genotypes were found to have less than 0.50% or equal, thirty-five bean genotypes were found to have between 0.51 to 1.00% and thirty-six bean genotypes were found to have between 1.01 to 3.00% Ca content under chilling stress (Table 3).

Table 3. Classification of bean genotypes according to Ca content under chilling stress

0.50% ≤ Ca content	0.51-1.00% Ca content	1.01-3.00% Ca content
Bn-2	Bn-6	Bn-10
Bn-4	Bn-8	Bn-12
Bn-15	Bn-9	Bn-13
Bn-17	Bn-16	Bn-14
Bn-27	Bn-24	Bn-18
Bn-29	Bn-28	Bn-19
Bn-32	Bn-30	Bn-20
Bn-56	Bn-33	Bn-22
Bn-57	Bn-34	Bn-23
Bn-63	Bn-36	Bn-26
Bn-65	Bn-39	Bn-35
Bn-74	Bn-40	Bn-37
Bn-75A	Bn-50	Bn-38
Bn-79	Bn-51	Bn-42
Bn-89	Bn-54	Bn-44
Bn-99	Bn-60	Bn-48
Bn-103	Bn-61	Bn-49
Bn-105	Bn-62	Bn-52A
Bn-106	Bn-64	Bn-52B
Bn-112	Bn-68	Bn-55
Bn-131	Bn-69	Bn-66
Bn-151	Bn-72	Bn-67
Bn-152	Bn-75C	Bn-70
Bn-154	Bn-77	Bn-71
	Bn-87	Bn-75
	Bn-104	Bn-76
	Bn-111	Bn-101
	Bn-147	Bn-102
	Bn-153	Bn-114
	Bn-157A	Bn-116
	Bn-157B	Bn-127
	Bn-158	Bn-130
	Bn-159	Bn-138
	Bn-162	Bn-139
	Bn-163B	Bn-140
		Bn-163A

4. Conclusions

Chilling stress has been shown to affect Ca content in common bean genotypes. Despite the chilling stress, it can be suggested that the genotypes, which have the best Ca content (Bn-114, Bn-13, Bn-37, Bn-163A, Bn-35) can be used in breeding for chilling tolerant cultivars. There is a need for in-depth understanding of chilling stress and Ca interactions due to limited literature about the calcium content of beans. For this reason, our work highlights the chilling and Ca content relations for future studies.

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