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The Effects of $CaCl_2$ and KCl Concentrations on Chilling Resistance of Bean (*Phaseolus vulgaris* L.)

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ÖZET: Bu araştırmada fasulye bitkisinin vejetatif organlarının (kök, gövde ve yaprak) soğuğa dayanıklılığı üzerine $CaCl_2$ ve KCl (0.025, 0.05 and 0.1 M)'nin etkileri kontrollü üşüme testleriyle araştırıldı. Fideler, sulama suyuna $CaCl_2$ ve KCl ilave edildikten sonra 1 gün boyunca aklime (15^0C ışık: 10^0C karanlık,14 saat ışık: 10 saat karanlık) edildi. Daha sonra fideler 9 gün boyunca üşüme sıcaklıklarına (CTs) ($10/5^o$C) maruz bırakıldı. 0.05 M KCl ve 0.025 M $CaCl_2$ gövde uzamasını teşvik ettiği belirlendi. Nisbi Kuru ağırlık (RDW) genellikle kök ve gövdede tüm $CaCl_2$ ve KCl uygulamaları tarafından azaltıldı, ama RDW bu uygulamalar tarafından yaprakta artırıldı (0.05 M KCl hariç). 0.1 M $CaCl_2$ uygulaması ise RDW'ı bütün organlarda artırdı. 0.1 M $CaCl_2$ uygulamasının her üç organda çözünebilir protein miktarını (SPtC) artırdığı belirlendi. Proline içeriği (PlC) bazı uygulamalar tarafından artırılırken, bazıları tarafından azaltıldı. Gövde canlılığının tüm uygulamalar tarafından azaltılmasına karşın, bazı uygulamalar (kök için 0.025 M KCl, yaprak için 0.025 M KCl, 0.1 and 0.05 M $CaCl_2$) tarafından kök ve yaprak canlılığı artırıldı. Biz, bu araştırmada, sağlanabilen direnç cok az olsa bile, belirli KCl ve $CaCl_2$ (0.025 M KCl, 0.025 M KCl, 0.1 ve 0.05 M $CaCl_2$) uygulamalarının üşümeye hassas olan fasulyeye direnç sağlamada kullanılabileceğini önermekteyiz.

Anahtar Kelimeler: Üşümeye direnç, Canlılık, KCl, $CaCl_2$, Fasulye, *Phaseolus vulgaris*

ABSTRACT: The effects of $CaCl_2$ and KCl on the cold hardiness of bean (*Phaseolus vulgaris* L. cv. Terzi Baba) was investigated using controlled chilling tests in vegetative organs (the root, the stem, the leaf). The seedlings were acclimated (15^0C light: 10^0C dark under a 14-h photoperiod) for 1 day after $CaCl_2$ and KCl was applied via the irrigating water. Then the seedlings were exposed to chilling temperatures (CTs) ($10/5^o$C) for 9 days. 0.05 M KCl and 0.025 M $CaCl_2$ induced in the stem length. Relative dry weight (RDW) was generally decreased by all $CaCl_2$ and KCl treatments in the root and the stem, but RDW was promoted by these treatments (except from 0.05 M KCl) in the leaf. 0.1 M $CaCl_2$ increased level of RDW in the all organs. It was determined that 0.1 M $CaCl_2$ increased SPtC in three organs. Proline content (PlC) was increased by some of treatments, while it was decreased by others. Although the stem viability was decreased by all treatments, the root and the leaf viability was increased by some treatments (0.025 M KCl for the root, 0.025 M KCl, 0.1 and 0.05 M $CaCl_2$ for the leaf) as compared to control. We suggest here that certain concentrations of suplemental KCl and $CaCl_2$ (0.025 M KCl, 0.025 M KCl, 0.1 and 0.05 M $CaCl_2$) can be use for supplying of chilling resistance to plants naturally sensitive against to chilling, even if the resistance is a very low levels.

Key Words: Chilling resistance, Viability, $CaCl_2$, KCl, Bean, *Phaseolus vulgaris.*

INTRODUCTION

Higher plants from thermally contrasting habitats show considerable differences in physiological and biochemical parameters. Capacity of plants for adaptations to different temperatures may be due to variation in key components of cellular constituents which enable plants to work efficiently under various temperature regimes (De *et al*., 1996). Accumulation of proline in plants during various environmental stress is a well known fact (Lalk *et al*., 1985; Machakova *et al*., 1989) and used as an index of stress resistance (De *et al*., 1996).

It has been reported that many environmental and hormonal signals (touch, wind, gravity, light, cold, auxin, gibberellic acid, Abscisic acid, salt, fungal elicitors) induce changes in cytosolic Ca^{++} levels that preceed the physiological responses (Poovaiah and Reddy, 1993). Thus, the impact of environmental and biotic stresses on plants can be mediated by cytosolic Ca^{++}. For example, it has been proved such a role of Ca in cold acclimation (Dhindsa and Monroy, 1994; Monroy *et al*, 1993).

The compatible osmotic solutes that generally accumulate during environmental stresses may be triggered by membrane bound signal tranducers. The Ca^{++} appears to be the most likely mediator, which was shown to link the extracellular stimuli with the intracellular environment (Uma Shaankar, 1985). In addition, there is ample evidence for the role of Ca in plant growth and development and in maintenance and modulation of various cell functions (Leonard and

Hepler, 1990; Poovaiah and Reddy, 1993). This evidence is based on function as well as in cell wall structure. For example, it is essential to have Ca^{++} in the extracellular solution to ensure the maintenance of selective permeability, i.e., membrane integrity. It is also well known that Ca^{++} is an integral part of the cell wall where it provides stable, but reversible, intramolecular linkages between pectic molecules, resulting in cell wall rigidity. Moreover, Ca^{++} stabilizes cell membranes by bridging phosphate and carboxylate groups of phospholipids at the memrane surface (Legge *et al*., 1982). Presence of extracellular Ca^{++} increases bonds between the cell wall and plasma membrane (Gomez-Lepe *et al*., 1979). A wide-range of extracellular signals such as cold and heat stresses have been shown to cause transient elevation of cytosolic free Ca^{++} level, and the concentration of Ca2+ returns to the resting level, which requires active pumping of Ca^{++} to the apoplast or organelles to maintain Ca^{++} homeostasis (Knight, 2000). Interestingly, Ca^{++} is a nontoxic mineral nutrient and plant cells can tolerate high concentrations of extracellular Ca^{++} (Palta and Lee-Stadelman, 1983). Furthermore, Ca^{++} has regarded as an important intracellular secondary messenger (Poovaiah and Reddy, 1993). Various studies have provided strong evidence implicating the regulation of various cell functions by cytosolic (free) Ca^{++} concentration. These studies suggest that Ca^{++} is a messenger in transducing external stimuli in plants. These signals often use plasma membrane-associated protein kinases, phosphadidylinositol patways, or both (Poovaiah and Reddy, 1987).

On the other hand, freezing injury results in increased efflux of ions from plant tissues (Palta *et al*., 1977a, 1977b). Potassium (K) is the major cation that leaks out of cells (Palta *et al*. 1977a). From these results, it was suggested that alteration in the K^+ permeability of the cell membranes was an early symptom of freeze-thaw injury (Palta and Li, 1978, 1980). In a follow up study, Arora and Palta (1989) demonstrated that K^+ efflux rate following freezing-thaw injury is markedly reduced in the presence of extracellular Ca^{++}. For example, in freeze-thaw-injured onion (*Allium cepa* L.) bulb cells, K efflux rate was reduced by half in the presence of 25 mM $CaCI_2$, whereas equivalent concentrations of NaCI had no effect on K^+ efflux. In addition, it has been demonstrated that exogenously KCI negatively effected the cold hardiness in onion bulb cells, and the development of these symptoms by KCI could be prevented by adding 10 to 20 mM $CaCI_2$ to medium (Arora and Palta, 1986).

Although the effects of Ca and K are well known in level cell, there is a few researches on the effects of the nutrient elements on chilling resistance at the whole plant body and especially its organs.

Consequently, we aimed here to determine and compare effects of exogenous $CaCI_2$ and KCI at three different concentrations (0.025, 0.05 and 0.1 M) on the root and the stem lengths, relative dry weigth (RDW), proline and protein contents, organs' viability of the bean plants (*Phaseolus vulgaris* L**.** cv. Terzibaba) at chilling temperatures (CTs, 10/5 ^oC) and thus, research that whether treatments of these elements cause to chilling resistance in the plant organs (the root, the stem, the leaf) or not.

MATERIALS and METHODS

Plant material

Bean Terzi Baba 98 was used in this study. The experiments were replicated a minimum of three times. Seedlings were grown in 3-L glazed pots with approximately 10 seedlings per pot in sand. Initially seeds were sprouted at 20 ^oC the dark. Seedlings were grown at 25 ^oC light: 20 ^oC dark under a 16-h photoperiod controlled-environment chamber. Acclimation occured at 15 ^oC light: 10 ^oC dark under a 14-h photoperiod for 1d. Then, the plants were exposed to 10 ^oC light: 5 ^oC dark under a 12-h photoperiod until leaves were wrinkled (9 d). We used two different control groups: 1. NCS: Seedlings which had been grown under normal conditions (25/20^oC), without Ca and K application, 2. Control: Seedlings which had been grown at cold conditions (10/5^oC) after the acclimation and without Ca and K application.

Application of Ca and K

The $CaCI_2$ and KCI (0.025, 0.05 and 0.1 M) were applied to the plants as irrigating water (Gusta *et al*., 1982), before they were exposed to cold acclimation. To the control plants were applied to deionized water in the same volume. It was applied to the normal conditions seedlings (NCS) just deionized water and they were grown 25^oC light: 20^oC dark under a 14-h photoperiod.

Relative dry weight (RDW) content

After chilling treatment, plants were cut into the root, stem and leaf and sampled randomly for each treatment group. Almost 1 g of the samples were then dried at 80 ^oC for 48 h in a forced-air drying oven. RDW content was expressed as "g dry weight per 100 g fresh tissue".

Measurement of viability with TTC test

A certain amount of the samples (0.5 g) were cut into small pieces (1-cm sections) for the roots and the stems for each different treatment. However, it was prepared special samples from the leaves for the TTC test. It was taken 1-cm diameter discs (10 discs) from the leaves. After 0,5-g the pieces of organ and the leaf discs were put in test tubes, they were washed with deionized water 2-3 times and filtered. 0,05 % TTC (4mL) solution was added to each tube. Incubation was made at 25 ^{o}C for 18 h on shaker. At the end of the time, liquid with red colour (formazon) in the tubes was filtered by Whatman 42 filter paper. Absorbance at 485 nm of formazon was recorded for each tube (modified from Steponkus and Lanphear, 1967; Towill and Mazur, 1975).

Soluble protein content (SPtC)

The samples (0.5 g) separetely taken from the roots, the stems and the leaves for each treatment were analyzed as coomassie brillant blue method (Bradford, 1976). Results were expressed as "mg protein/ g fresh tissue".

Proline content (PlC)

Preparing of the samples from each treatment was made as soluble protein content analyses mentioned above. Proline content analysis were made according to acid-ninhydrin method (Bates, 1973). Results were expressed as "μ g proline/ g fresh tissue".

Statistical Analysis

Data were analysed, by Analysis of Variance using the SPSS (1985). Duncan Multiple Range Test was used for comparison of treatments.

RESULTS

The effects of Ca and K on RDW

As a result of treating chilling temperatures (10/5 ^{o}C) for 9 days, relative dry weight (RDW) was increased by 0.1 M and 0.05 M $CaCI_2$, 0.1 M KCI treatments in the leaf, but all treatments decreased it in the root and the stem (except from 0.1 M $CaCI_2$) as compared with control (Table 1.). The highest RDW value was obtained from 0.1 M $CaCI_2$ in three organs.

Table 1. The effects of Ca and K on RDW at CTs

Treatments	RDW (g dry weight/ 100 g fresh tissue)		
	root	stem	leaf
NCS (25/20 ^{o}C)	5.47^{b}	6.40^{d}	8.87^{f}
Control (10/5 ^{o}C)	6.53^{a}	8.20^{b}	10.40^{de}
0.025 M $CaCI_2$ (10/5 ^{o}C)	5.30^{b}	8.30^{b}	10.80^{cd}
0.05 M $CaCI_2$ (10/5 ^{o}C)	5.13^{bc}	7.80^{bc}	12.40^{b}
0.1 M $CaCI_2$ (10/5 ^{o}C)	7.00^{a}	9.50^{a}	14.40^{a}
0.025 M KCI (10/5 ^{o}C)	4.30^{c}	7.10^{cd}	10.60^{cd}
0.05 M KCI (10/5 ^{o}C)	4.70^{bc}	7.00^{cd}	9.50^{ef}
0.1 M KCI (10/5 ^{o}C)	4.30^{c}	6.30^{d}	11.57^{bc}

* Means in the same column followed by the same letter are not significantly different at the ($P<0.05$) level
NCS: Normal conditions seedlings RDW: Relative dry weight

The effects of Ca and K on the organs' viability

It was determined that the root viability was the only increased by 0.025 M KCI treatment(Table 2.). Although all treatments decreased viability in the stem, some treatments (0.025 M KCI, 0.1 and 0.05 M $CaCI_2$) increased it in the leaf as compared with control.

Table 2. The effects of Ca and K on organs' viability at CTs

Treatments	Viability (A 485 values)		
	root	stem	leaf
NCS (25/20 °C)	0.598[c]	0.379[d]	0.117[e]
Control (10/5 °C)	1.021[b]	0.450[de]	0.272[cd]
0.025 M $CaCI_2$ (10/5 °C)	1.082[b]	0.520[cd]	0.412[a]
0.05 M $CaCI_2$ (10/5 °C)	0.925[b]	0.575[bc]	0.248[d]
0.1 M $CaCI_2$ (10/5 °C)	1.520[a]	0.520[cd]	0.240[d]
0.025 M KCI (10/5 °C)	0.852[bc]	0.602[bc]	0.221[d]
0.05 M KCI (10/5 °C)	1.735[a]	0.813[a]	0.330[b]
0.1 M KCI (10/5 °C)	1.078[b]	0.621[b]	0.312[bc]

* Means in the same column followed by the same letter are not significantly different at the ($P<0.05$) level
NCS: Normal conditions seedlings
Note: The lowest values are showing that the viability is more than the highest values.

The effects of Ca and K on the soluble protein content (SPtC)

The SPtC was statistically increased in the control as compared with NCS, but $CaCI_2$ and KCI treatments statistically decreased it much more (except from 0.025 M KCI for the root, 0.025 M $CaCI_2$ and 0.05 M KCI for the stem) as compared with control (Table 3.).

Table 3. The effects Ca and K on the SPtC at CTs

Treatments	SPtC (mg protein/g fresh tissue)		
	root	stem	leaf
NCS (25/20 °C)	9.58[d]	9.10[e]	53.30[d]
Control (10/5 °C)	12.07[b]	15.87[bc]	73.20[a]
0.025 M $CaCI_2$ (10/5 °C)	8.37[e]	19.70[a]	58.60[c]
0.05 M $CaCI_2$ (10/5 °C)	10.90[c]	13.87[d]	64.40[b]
0.1 M $CaCI_2$ (10/5 °C)	10.30[cd]	14.60[cd]	57.50[c]
0.025 M KCI (10/5 °C)	15.67[a]	13.37[d]	62.73[b]
0.5 M KCI (10/5 °C)	7.80[e]	16.27[b]	53.93[b]
0.1 M KCI (10/5 °C)	8.30[e]	13.73[d]	38.00[e]

* Means in the same column followed by the same letter are not significantly different at the ($P<0.05$) level
NCS: Normal conditions seedlings SPtC: Soluble Protein Content

The effects of Ca and K on the proline content (PlC)

Chilling temperatures (CTs) increased PIC in organs of control seedlings as compared with NCS, but PIC was increased by some of $CaCI_2$ treatments (0.025 M $CaCI_2$ and 0.05 M KCI for the root, 0.025, 0.05 and 0.1 M $CaCI_2$, 0.05 and 0.1 M KCI for the stem, 0.1 M $CaCI_2$ and 0.05 M KCI for the leaf) and decreased by others as compared with control (Table 4.).

The effects of Ca and K on the root and stem length

None of $CaCI_2$ and KCI treatments statistically effected in the root length of seedlings as compared with control (Table 5.). It was just determined that 0.05 M KCI and 0.025 M $CaCI_2$ increased the stem length.

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Table 4. The effects and Ca and K on the PlC at CTs

Treatments	PlC (µg proline/g fresh tissue)		
	root	stem	leaf
NCS (25/20 °C)	151[e]	588[e]	741[f]
Control (10/5 °C)	226[c]	1207[d]	1662[c]
0.025 M $CaCI_2$ (10/5 °C)	449[a]	1366[c]	1450[e]
0.05 M $CaCI_2$ (10/5 °C)	70[f]	2257[b]	1529[de]
0.1 M $CaCI_2$ (10/5 °C)	48[g]	1370[c]	1837[b]
0.025 M KCI (10/5 °C)	27[h]	646[e]	1612[cd]
0.05 M KCI (10/5 °C)	276[b]	2459[a]	1936[a]
0.1 M KCI (10/5 °C)	173[d]	1458[c]	1447[e]

* Means in the same column followed by the same letter are not significantly different at the (P<0.05) level
NCS: Normal conditions seedlings PlC: Proline Content

Table 5. The effects of Ca and K on the root and the stem length at CTs

Treatments	milimeters / seedling	
	root	stem
NCS (25/20 °C)	16.1[a]	36.9[a]
Control (10/5 °C)	13.2[b]	24.8[bc]
0.025 M $CaCI_2$ (10/5 °C)	13.7[b]	26.0[b]
0.05 M $CaCI_2$ (10/5 °C)	13.1[b]	23.2[bc]
0.1 M $CaCI_2$ (10/5 °C)	12.9[b]	22.6[bc]
0.025 M KCI (10/5 °C)	12.6[b]	21.2[cd]
0.05 M KCI (10/5 °C)	13.6[b]	27.0[b]
0.1 M KCI (10/5 °C)	13.1[b]	22.1[bc]

* Means in the same column followed by the same letter are not significantly different at the (P<0.05) level
NCS: Normal conditions seedlings

DISCUSSION

In this study, although relative dry weight (RDW, g dry weight/100 g fresh tissue) was generally decreased by all $CaCI_2$ and KCI treatments in the root and the stem, RDW was promoted by the all treatments (except from 0.05 M KCI) in the the leaf to compared with control(Table 1.). It has been reported that plants receiving Ca under heat stress had significantly higher total leaf and total dry weights compared to plants with no supplemental Ca under identical conditions (Palta, 1996). Despite we didn't work on total dry weight under heat stres in this research, we also found that seedlings which had been applied $CaCI_2$ (0.025, 0.05 and 0.1 M) had significantly higher RDW in the leaf to compared with plant's leaves of control and NCS under chilling stress (Table 1.). These results mean that suplemental Ca due to indirectly increment of dry subtances in plants and/or removing of water from the leaves. It has already been reported that in plant water content diminishes during cold acclimation, and the decreasing of water content due to accumulation of abscicic acid (ABA), and ABA caused synthesis of new many kind of special proteins (Chen *et al*.1983; Guy and Haskell, 1988).

We determined that 0.025 M KCI increased viability in the most range in the leaf and the root, and other KCl treatments diminished it in the stem as compared with control (Table 2). It was interesting that we determined the positively effect of 0.025 M KCI on the leaf and the root viability. Because it has been reported that K retards the cold hardening process of Scots pine (Christersson, 1975; Aronson, 1980). In a related study, it was proposed that chilling injury results when extracellular K^+ removes Ca^{++} from the outer face of the plasma membrane (Palta, 1996). In

this way, our the finding in a related with 0.025 M KCI isn't accord with previous works. However, Palta and Li (1980) suggested that cellular symptom results from the secondary injury caused by a high concentration of extracellular K^+. We also determined that viability of the three organ were negatively effected from by high concentrations of KCl (0.05 and 0.1 M). We propose that exogenous KCI may increase chilling resistance at low concentrations in bean, but not in high concentrations. On the other hand, it was informed that chilling injury results in increased efflux of ions from plant tissues (Palta *et al*., 1977a, 1977b). K^+ is the major cation that leaks out of cells (Palta *et al*. 1977a). From these results, it was suggested that alteration in the K^+ permeability of cell membranes was an early symptom of freeze-thaw injury (Palta and Li, 1978, 1980). In a other following study, Arora and Palta (1989) demonstrated that K^+ efflux rate following freeze-thaw injury is markedly reduced in the presence of extracellular Ca^{++} (20 mM $CaCl_2$) in onion (*Allium cepa* L.) bulb cells. Salycilic acid-induced heat or cold tolerance in grape leaves is through Ca2+ homeostasis and a higher activity of antioxidant systems that is correlated to the cytoplasmic Ca2+ increase in cells under heat or cold stres (Wang and Li, 2006). We determined that 0.025 M $CaCl_2$ negatively effected viability in the three organs by TTC test to compared with control (Table 2.). Our this result obtained from organs of the bean plant is not confirm to the previous work's finding obtained from onion bulb cells. It may be thought that both of the results can be fact. Because results of the two studies had been obtained from different plant species and we studied effects of $CaCl_2$ in organ level, but the previous works have been made on tissue level. However, 0.025 KCl and 0.05 M $CaCl_2$(not statistically important for root) positively effected viability in the leaf and the root, and 0.1 M $CaCl_2$ increased it in the leaf, but the same concentrations (0.1 M) of KCI decreased it in the three organs (Table 2.). It was reported that Ca^{++} is a nontoxic mineral nutrient and plant cells can tolerate high concentrations of extracellular Ca^{++} (Palta and Stadelmann, 1983). Moreover, it has been known that the primary effect of abiotic and biotic stresses on plant tissues is the loss of cell membrane integrity, resulting among others, in the displacement of membrane bound calcium (Stadelman and Stadelman, 1976). The significance of such displaced calcium in cell metabolic regulation has been realized, after the discovery of the calcium-modulated proteins. A number of physiological processes such as the action of growth regulators and the activation of several enzyme systems have been shown to be regulated by calcium and calmodulin (Suresh *et al*., 1991). We also agree that exogenous Ca^{++} in high concentrations (0.05 and 0.1 M) is a nontoxic mineral nutrient and we proposed that $CaCl_2$ must be in high concentrations (>0.025 M) for it can be positively effective on chilling resistance in the leaf of bean.

It has been known that soluble protein content (SPtC) was increased by stress temperatures and cold acclimation in plants (Omran *et al*., 1971). In this study, we also determined the same result in with compared to seedlings growed in normal conditions (NCS, 25/20 ^{0}C) (Table 3.). Moreover, SPtC was lower in most of treatments applied $CaCl_2$ and KCI than control, but higher than NCS. We think that this results may be normal. Because we treated cold acclimation (15 ^{o}C light: 10 ^{o}C dark under a 14-h photoperiod the 1st day) to all treatments (except from NCS) before chilling temperatures (10/5 ^{0}C) period. On the other hand, it has been reported that levels of endogenous ABA increases during cold acclimation (Rigby *et al*., 1977) and ABA acts by activating the genetic system responsible for the freezing-tolerance response, and it causes many changes in pattern of protein synthesis (Mohapatra *et al*., 1988). However, it was interesting that $CaCl_2$ and KCI applied at three concentrations generally caused to decreasing in the SPtC in the three organs. But we just determined to increasing of SPtC in some of treatments (0.025 M KCI for the root, 0.05 M KCI, 0.025 M $CaCl_2$ for the stem) to compared with control (Table 2.). These results mean that there is naturally differences in SPtC between organs of bean (maybe in all plants), and SPtC can increase in different levels as depend upon organs at chilling temperatures, and suplemental $CaCl_2$ ve KCI may effects its level negatively or positively.

It was shown that in the treatments increased viability (0.05 M and 0.1 M $CaCl_2$ and 0.025 M KCI for the leaf). But they didn't increase SPtC in the same rate. In addition, 0.025 M $CaCl_2$ and 0.05 M KCI increased SPtC, although they markedly decreased viability on the stem (Table 2, 3). These results signify that SPtC increment may be necessary for chilling resistance processes, but it doesnt mean that increment suplies chilling resistance in any case. It has been already known that abscisic acid induced by chilling causes many changes in pattern of protein synthesis and supplies synthesis of specific proteins (Mohapatra *et al*., 1988). This is why, we suggest here it may be more important quality of SPtC than its quantity for chilling resistance.

We determined that chilling temperatures caused to increase proline content (PIC) in three organs (Table 4.). This result agree with previous studies (Yelenosky, 1979; Purvis, 1981; Wang, 1982; Duncan and Wildholm, 1987; Kushad and Yelenosky, 1987). PIC increased/ or decreased different levels in the between organs at all treatments as like SPtC (Table 3, 4.). In addition, it was determined that treatments of 0.05 and 0.1 M $CaCI_2$ and 0.025 M KCI was due to increment both in viability and PIC level (nearly 2.5 fold) in the leaf. But, the all $CaCI_2$ and KCI treatments increased PIC levels caused to decreasing of viability in the root and the stem (Table 2, 4.). Wang (1982) has reported that it was not clear if proline accumulation was a consequence of chilling stress or was an essential part of the hardening mechanism. We suggested that it can be an increment/or decreasing of proline accumulation as a consequence of chilling stress, but the increment or decreasing isn't certainly a sign of chilling resistance in bean.

The results obtained from this study demonstrated that it is generally ineffective the role of three concentrations (0.025, 0.05 and 0.1 M) of $CaCI_2$ and KCI on elongations of bean plants at chilling temperatures (CTs). It was interesting that only 0.05 M KCI and 0.025 M $CaCI_2$ statistically increased the stem length as compared with control (Table 5.). It has been reported that the addition of potassium under controlled growth conditions or by fertilizing in the field may prolong the growth of tree (Sarjala *et al*., 1997). The effect of 0.05 M KCI accords with finding of Sarjala *et al*. (1997), but not with our the other concentrations.

CONCLUSIONS

1. 0.025 KCl and 0.05 M $CaCI_2$ positively effected viability in the leaf and the root (not statistically important for root), and 0.1 M $CaCI_2$ the only increased it in the leaf, but the same concentrations (0.1 M) of KCI decreased it in the three organs
2. Exogenous Ca^{++} in high concentrations (0.05 and 0.1 M) is a nontoxic mineral nutrient for bean plant, as contrast to KCl.
3. Increment of SPtC may be necessary for occuring of chilling resistance processes, but it doesnt mean that the increment suplies chilling resistance in any case.
4. It can be an increment/or decreasing of proline accumulation as a consequence of chilling stres, but the increment or decreasing isn't certainly a sign of chilling resistance in bean. However, change of proline level can be an indicator implying that plant had been exposed to cold and/or another kind of stress.
5. The three concentrations (0.025, 0.05 and 0.1 M) of $CaCI_2$ and KCI has generally no effect on elongations of bean plants' stems and especially roots at chilling temperatures.

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REFERENCES

Aronson, A., 1980. Frost hardiness in Scots pine (*Pinus sylvestris* L.*)* II. Hardiness during winter and spring in young trees of different mineral nutrient status. Stud. For. Suec. 155:1-27.

Arora, R. and J.P. Patla, 1986. Protoplasmic swelling as a symptom of freezing injury in onion bulb cells. Its stimulation in extracellular KCI and prevention by calcium. Plant Physiol., 82: 625-629.

Arora, R.and J.P. Palta, 1989. Perturbation of membrane calcium as a molecular mechanism of freezing injury, p. 281-290. In, J.H. Cherry (e.d.). Environmental stress in plants. NATO Advanc. Sci. Inst. Series, G 19. Springer–Verlag, Berlin.

Bates, L.S. 1973. Rapid determination of free proline for water-stress studies. Plant and soil., 39: 205-207.

Bradford, M.M. 1976. A rapid and sensitive method for the quantition of microgram quantities of protein utilising the principle of protein-dye binding analitical. Biochem.,72: 248-254.

Chen, H.H., P.H. Li and M.L. Brenner, 1983. Involvement of abscisic acid in potato cold acclimation. Plant Physiol. 71: 362-365.

Christersson, L., 1975. Frost-hardiness development in *Pinus silvestris* L.seedlings. Stu. For. Sue., 103:1-27.

De, B., S. Bhattacharjee and A.K. Mukherjee, 1996. Short therm heat shock and cold shock induced proline accumulation in relation to calcium involvement in *Lycopersicon esculentum* (Mill) cultured cells and seedlings. Indian J. Plant Physiol., New Series 1(1): 32-35.

Dhindsa, R.S.and A.F. Monroy, 1994. Low temperature signal transduction, gene expression, and cold acclimation, Multiple roles of low temperature, p. 501-514. In, J.H. Cherry (e.d.). Biochemical and cellular mechanism of stress tolerance in plants. NATO Advanced Sci. Inst. Series, H 86. Springer-Verlag, Berlin.

Duncan, R.D. and J.M. Widholm, 1987. Proline accumulation and its implication in cold tolerance of regenerable maize callus. Plant Physiol., 83: 703-708.

Gomez-Lepe, B.E., O.Y. Lee-Stadelmann, J.P. Palta, and E. J.Stadelmann, 1979. Effects of octylguanidine on cell permeability and other protoplasmic properties of *Allium cepa* epidermal cells. Plant Physiol., 64: 131-138.

Gusta, L.V., D.B. Fowler, and N..J. Taylor, 1982. The effect of abscisic acid and cytokinins on the cold hardiness of winter wheat. Can. J.Bot., 60: 301-305.

Guy, C.L. and D. Haskell, 1988. Detection of polypeptides associated with the cold acclimation process in spinach. Electrophoresis, 9: 787-796.

Knight, H., 2000. Calicium signaling during abiotic stress in plants, Int. Rev. Cytol. 195: 269–324.

Kushad, M.M. and G., Yelenosky, 1987. Evaluation of polyamine and proline levels during Low temperature acclimation of citrus. Plant Physiol., 84: 692-95

Lalk, I. and K. Dorfling, 1985. Hardening, ABA, proline and freezing resistance in two winter wheat varieties. Physiol. Plant., 63: 287-292.

Leonard, R.T.and P.K. Hepler, 1990. Calcium in plant growth and development. Amer. Soc. Plant Physiol., Rockville, Md.

Machakova, I., A. Hansiova and J. Krekule, 1989. Levels of ethylene, ACC, MACC, ABA and Proline as an indicator of cold hardening and frost resistance in winter wheat. Physiol. Plant., 76: 603-607.

Mohapatra, S.S., R.J.Poole and R.S.Dhindsa, 1988. Abscisic acid-Regulated gene expression in relation to freezing tolerance in *Alfalfa*[1]. Plant Physiol., 87: 468-473.

Monroy, A.F., F. Sahran and R.S. Dhindsa, 1993. Cold-induced changes in freezing tolerance, protein phosphorilation, and gene expression. Plant Physiol., 102: 1227-1235.

Omran, R.G., C.R. Benedict and R.D. Powell, 1971. Effects of chilling on protein synthesis and CO_2 fixation on cotton leaves. Crop Science., 11: 554-556.

Palta, J.P., J. Lewitt and E.J. Stadelmann, 1977a. Freezing injury in onion bulb cells, I. Evaluation of the conductivity method and analiysis of ion and sugar efflux from injured cells. Plant Physiol., 60: 398-402.

Palta, J.P., J. Lewitt and E.J. Stadelmann, 1977b. Freezing injury in onion bulb cells, a comparison of freezing vs. desiccation and living vs. dead cells. Plant Physiol., 41: 273-279.

Palta, J.P. and Lee-Stadelmann, E.J., 1983. Vacuolated plant cells as ideal osmometer: Revesibility and limits plasmolsis, and estimation of protoplasm volumme in control and water-stress-tolerant cells. Plant, Cell Environ., 6:601-610.

Palta, J.P.and P.H. Li, 1978. In plant cold hardiness and freezing stress-mechanism and crop implications. Edited by P. H. Li and A. Sakai (Acad. press, New York). p.93-115.

Palta, J.P. and P.H. Li, 1980. Alterations in membrane transport properties by freezing injury in herbaceous plants. Evidence against rupture theory. Physiol. Plant. 50: 169-175.

Palta, J.P., 1996. Role of calcium in plant responses to stresses, Linking basic research to the linking basic research to the solution of practical problems. Hortscience, 31(1): 51-57.

Poovaiah, B.W. and A.S.N.Reddy, 1987. Calcium messenger systems in plants. CRC Crit. Rev. Plant Sci., 6, 47-102.

Poovaiah, B.W. and A.S.N. Reddy, 1993. Calcium and signal transduction in plants. CRC Crit. Rev. Plant Sci., 12(3), 185-211.

Purvis, A.C., K. Kawada, and W. Grierson, 1979. Relationship between midseason resistance to chilling injury and reducing sugar level in grapefruit peel. HortScience, 14: 227-229.

Rigby, P.W.J., M. Dieckmann, C. Rhodes and P. Berg, 1977. Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase. I. J. Mol. Biol., 113: 237-251.

Sarjala, T., K. Taulavuori, E. Maija and A.B. Edfast, 1997. Does Availability potassium affect cold hardening of Scots pine through polyamine metabolism? Physiol. Plant, 99:56-62.

Stadelman, O.Y. and E.J. Stadelman, 1976. In water and plant life, proplems and modern approaches, edited by O L Lange, L Kappen &E D Schulze (Springer- Verlag, Berlin), 268.

Steponkus, P.L. and F.O. Lanphear, 1968. The relationship of carbohydrates to cold acclimation of *Hedera helix* L. cv. Thorndale. Physiol. Plant., 21: 777-91.

Suresh, P., Lalitha, K.R., Udaya Kumar, M., Uma Shaanker, R., 1991. Involvement of calcium in abscisic acid, KCL, NaCL and moisture stress induced accumulation of proline in finger millet, *Eleusine coracana*. Indian J. of Exp.l Biol., 29: 355.

Uma Shaanker, R., 1985. Moisture stress induced loss of membrane integrity, accumulation of proline and their inter-relationship, Thesis, Univ. of Agric. Science, Bangalore, India,

Wang, C.Y., 1982. Physiological and Biochemical responses of plants to chilling streess. HortScience, 17 (2): 173-185.

Wang, L.J. and S.H. Li, 2006. Salicylic acid-induced heat or cold tolerance in relation to Ca2+ homeostasis and antioxidant systems in young grape plants. Plant Science 170: 685–694.

Yelenosky, G., 1979. Accumulation of free proline in citrus leaves during cold hardening of young trees in controlled temperature regimes. Plant Physiol., 64: 425-27.

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