

A STUDY ON THE RELATION OF COLD ACCLIMATION AND HARDINESS TO MINERAL NUTRITIONAL AND BIOCHEMICAL FLUCTUATIONS OF THREE AGRICULTURAL FORMS OF *Brassica oleracea* L.

III. MINERAL NUTRIENTS

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ABSTRACT

In this article, the changes of phosphorus, sodium, potassium, calcium and nitrogen contents of three cabbage cultivars in relation with cold acclimation and hardiness have been examined.

The effect of cold on three agricultural forms of white head cabbage was investigated in different development stages. For this purpose, they were planted in different seasons and sampled during seedling and headed phases. While three cultivars planted in June froze, those planted in October did not, but chilled only. The increases of mineral salt levels during cold acclimation and hardiness have been accepted as related with this fact. Significant changes have generally been obtained from the headed stages of cultivars, especially that of phosphorus was observed in all cultivars. Sodium and potassium levels changed considerably in Bayraklı; total nitrogen, sodium and potassium in Samsun top cultivar and calcium in Yeşilköy cultivar.

The results of present study can be considered as indicators of some practical approaches, such as fertilizing, spraying and choosing varieties of cabbages for improvement and partly for all crop plants.

INTRODUCTION

Although KAWANA et al. (1964) explained that nutrient salts have an important role in plant growth, they were also found to be very important in development of cold hardening by SAKAI (1960). While LYNCH and GOLDWEBER (1956) stated that there was not any relationship between phosphorus levels and frost hardiness in

woody plants, LI, WEISER and VAN HUYSTEE (1966) found that inorganic fraction of phosphorus decreased and total and organic phosphorus increased.

It has also been stated that potassium was able to increase cold hardening by accelerating amino acid and carbohydrate synthesis (HAYDEN et al., 1969) but COOK and DUFF (1976) have found that potassium fertilization did not increase accumulation of carbohydrates and freezing tolerance in herbaceous plants. Although JOINER and ELLIS (1964) found that potassium nutrition did not change cold hardening in woody plants and suggested that cold hardening was increased through providing little nitrogen and much potassium and phosphorus (MARSHAL, 1969). TRESHOW (1970) also stated that high amounts of sodium and calcium increased low temperature injury. As seen, there is not an agreement on cold hardening effects of nutrient salts in woody and herbaceous plants.

The aim of this study is also to examine the interrelations between important nutrients of plant metabolism, such as sodium, potassium, calcium, phosphorus and total nitrogen and the physiological mechanism cold acclimation and hardiness in cabbage. There are no studies on nutrition and cold acclimation and hardiness relations in cabbage, and the results in literature on woody and herbaceous plants are contradictory. Moreover, it has been noticed that most of the studies regarding nutrient salts have been based on the principles of fertilization.

In order to get information useful for agricultural practice, it was also aimed to choose the hardy cultivars using the cabbage seeds which have been registered by Agricultural Research Institutes. Choosing cultivars in this way, we shall try to evaluate physiological parameters to be used in seed improvement. To obtain the results which can be evaluated clearly in a comparative system, the same experimental methods were examined on three cabbage cultivars.

This paper comprises a part of Doctorate thesis completed in 1979.

MATERIAL AND METHODS

Sowing the seeds, planting the seedlings and preparing the soil, sampling methods, sample preparation were presented in the previous paper (ÖNCEL, 1984).

METHODS

MINERAL SALT ANALYSIS

Dried and ground plant samples have been exposed to wet digestion for the determinations of sodium, potassium, calcium and phosphorus (KACAR, 1972a). The samples have been digested according to Kjeldahl method for total nitrogen determination (KACAR, 1972b).

TOTAL NITROGEN DETERMINATION

Ammonium ion was determined with an ammonium electrode by ionmeter.

SODIUM AND POTASSIUM DETERMINATIONS

Sodium and potassium were determined flamephotometrically (KACAR, 1972c, d).

CALCIUM DETERMINATION

Calcium was determined with a calcium electrode by ionmeter.

PHOSPHORUS DETERMINATION

Phosphorus was determined with a fluoride electrode by ionmeter.

All of the experiments were repeated at least four times and significant differences at 5 % level were considered as statistically important.

RESULTS

THE CHANGES IN TOTAL NITROGEN CONTENT

It has been found that, total nitrogen content in the frozen unaffected young head sample of Samsun top cultivar which was in acclimation stage showed an increase. In the hardiness stage, it has been found that total nitrogen content decreased in the frozen affected young head sample, but increased when compared with the controls. Nitrogen decreased during acclimation, as seen in the frozen affected aged head sample, as a result of being affected (Figure 1). This also shows that total nitrogen is effective in the development of acclimation of Samsun

top cultivar. Increase of total nitrogen in the frozen affected aged head sample, can be considered as a result of hardening. In the frozen unaffected young head sample, which is acclimation stage of Yeşilköy cultivar, total nitrogen content decreased. But it increased in the hardiness stage corresponding to affection, namely in the affected young head sample; but in the frozen affected aged head sample, it decreased. This shows that the senescence developed and accordingly the hardiness could not proceed (Figure 1). It has also been found that, total nitrogen content decreased in acclimation and hardiness stages of headed Bayraklı cultivar (Figure 1).

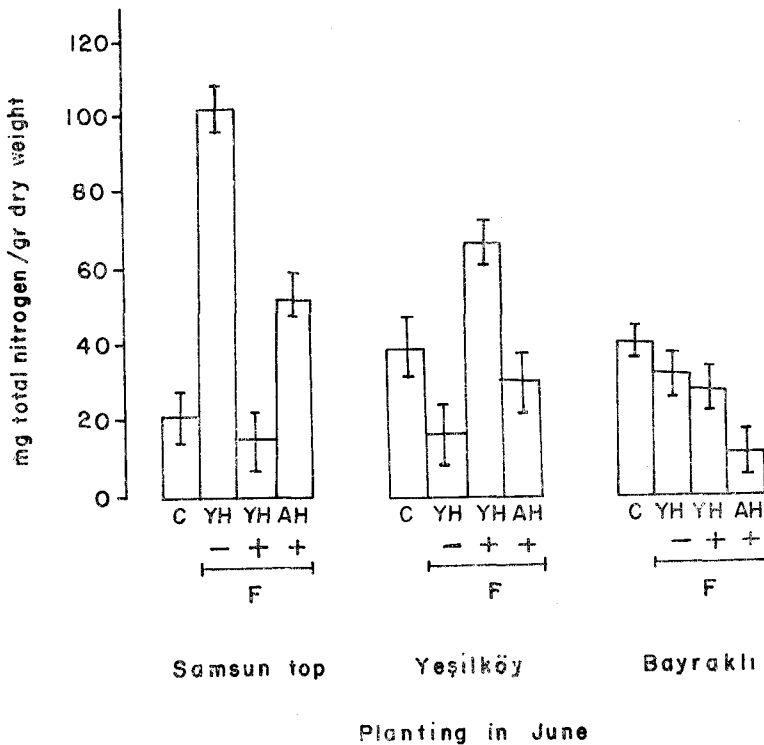


Figure 1. The content of total nitrogen in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

The total nitrogen content has also been found to be decreased in the chilled samples of the seedling cultivars (Figure 2).

The decrease in the content of total nitrogen, both in headed forms and seedlings of cultivars, is the result of senescence following aging. For, it is known that total nitrogen decreases during the senescence

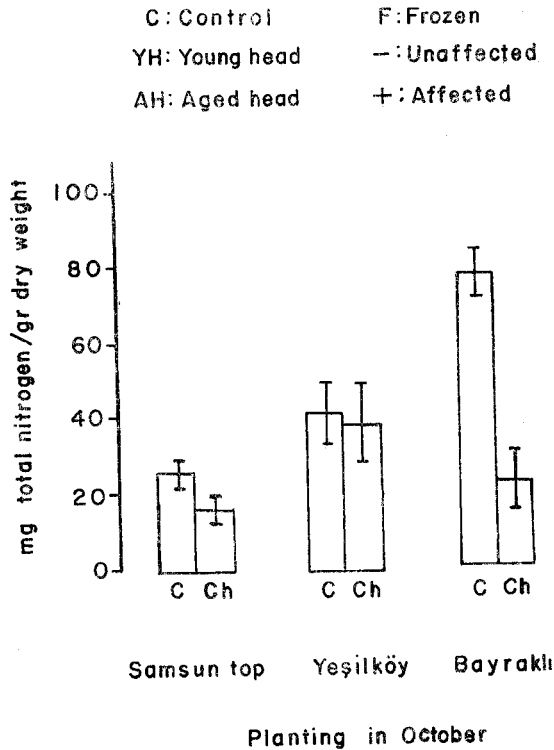


Figure 2. The content of total nitrogen in control and chilled samples of the seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

in plants (BEEVERS, 1968). Except for the increase of total nitrogen content only in the acclimation stage of the headed Samsun top cultivar and in the frozen affected young head sample of Yeşilköy cultivar, total nitrogen decreased in their other samples, Bayraklı cultivar and chilled samples in seedling stages. These developments can be explained in the light of findings of LE SAINT (1966), who explained that the decrease in total nitrogen, such as protein and amino acid nitrogen, increased the hardening. Moreover, it has been explained that, nitrogen levels always decreased during hardening (LAMB, 1967) and the nitrogen fertilization should also be decreased (GÖKGÖL, 1969). But there are reports, on the other hand, stating that the hardiness developed with the increase of nitrogenous compounds (COLEMAN, BULA and DAVIS, 1966). The studies on the increase (DROZDOV and SYCHEVA, 1965) and the decrease (HOWELL and JUNG, 1965) of the hardiness due to the high nitrogen nutrition, has shown that

the effect of nitrogenous compounds was contradictory to relation of nitrogen with cold acclimation and hardiness of plants. Our cultivars also showed that nitrogenous compounds had not been accumulated during acclimation and hardiness and their less quantities were sufficient for acclimation and hardiness. Unlike woody plants, the cabbage, a nondormant plant, keeping alive during the cold acclimation and hardiness periods, exhibits a very slow metabolism and according to our results, it consumes nitrogenous compounds, such as amino acid, protein and also total nitrogen.

THE CHANGES IN PHOSPHORUS CONTENT

During the cold acclimation and hardiness, increase of phosphorus playing an important role in plant metabolism is expected since the energy requirement for the hardiness development can be obtained from phosphorus containing compounds (VARDAR, 1972). In literature, the results which are related with phosphorus and cold hardiness seem to be insufficient and uncertain. However, during the cold acclimation and hardiness, it has been shown that the total and organic phosphorus increase and its inorganic form decrease (LI, WEISER and VAN HUYSTEE, 1966), but phosphorus nutrition generally increased the hardiness (LAMB, 1967; GÖKGÖL, 1969). In spite of contradictory results, the increase in phosphorus content for the acclimation and hardiness is logical: The active metabolism needed for the formation of the hardiness, and accordingly a rapid energy and phosphorus cycle are necessary. For this reason, during the cold acclimation and hardiness these relationships have been examined in this study.

In the frozen unaffected young head sample corresponding to the acclimation stage of Samsun top cultivar, the phosphorus content decreased, but in the frozen affected young and aged head samples which were hardening stages it reached to control level again (Figure 3). The phosphorus content has also decreased in the chilled samples of seedling Samsun top cultivar (Figure 4). The decrease of phosphorus content can be explained as a result of aging (GUARDIOLA and SUTCLIFFE, 1971). The decrease of the phosphorus content showed that the general metabolism was slow.

This fact has been observed in the chilled seedlings of Samsun top cultivar and in the frozen unaffected young head samples of the same cultivar when considered together with changes in soluble protein.

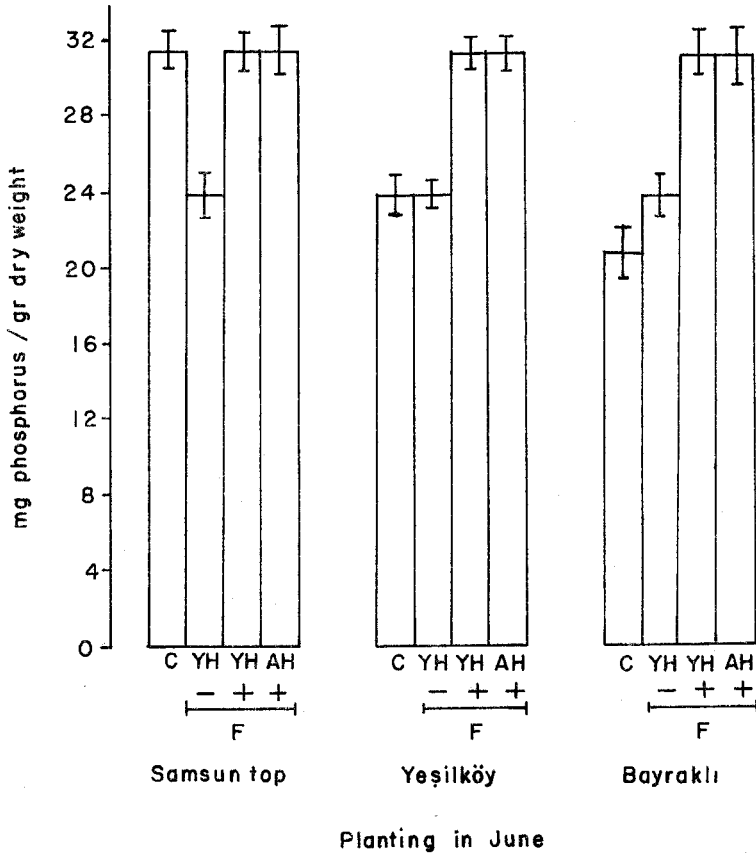


Figure 3. The content of phosphorus in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

But in the frozen affected young and aged head samples, the increase of phosphorus content is interesting while the soluble protein is decreasing. As a result, the Samsun top cultivar can acclimate in the cold and form the hardening capacity, but the hardiness could not be gained. In the hardiness period while the contents of glucose + saccharose, fructose + saccharose, total RNA and DNA and phosphorus are increasing, the steady level of soluble protein was the sign of this.

In the frozen unaffected young head sample which is acclimation stage of Yeşilköy cultivar, it has been found out that the phosphorus content has been at the control level. But in the frozen affected young and aged head samples which were in hardiness stages it has increased,

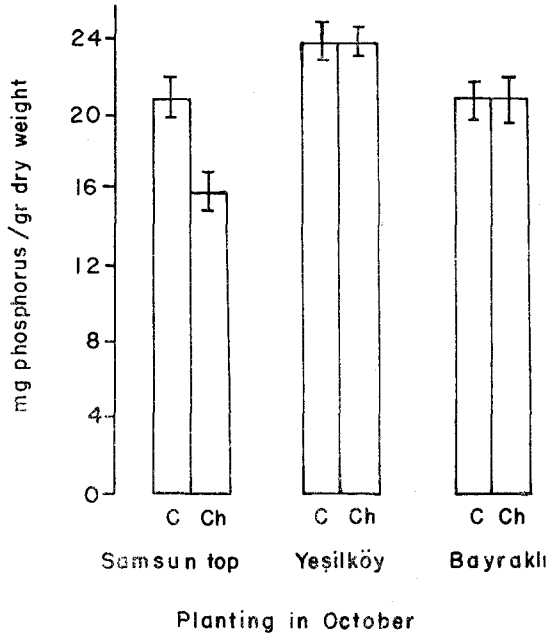


Figure 4. The content of phosphorus in control and chilled samples of the seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

and accordingly, this has been related with the hardiness (Figure 3). In the chilled seedling sample of Yeşilköy cultivar it has been found out that the phosphorus content has been at the same control level (Figure 4). Consequently, in both head and seedling samples which are in acclimation periods of Yeşilköy cultivar, the phosphorus content remained at the control level.

It is known that the phosphorus is a mobile element and is translocated from old leaves to young ones; namely, it decreases with aging (GUARDIOLA and SUTCLIFFE, 1971). No sign of significant growth during the hardiness, in other words, absence of the growth points to attract the phosphorus, accordingly its unchanged content, is normal. The increases of the total RNA and DNA, soluble protein, fructose + saccharose contents have shown that the Yeşilköy cultivar can acclimate. In hardiness stage, it has been observed that previously examined biochemical parameters also changed in such a way that they

could increase the hardness in parallel to the increase of the phosphorus content.

In this study, the total phosphorus changes have been examined. Only this parameter can not be considered as the sign of metabolic activity (GUARDIOLA and SUTCLIFFE, 1971). Yet, organic and inorganic phosphorus contents, their ratios and the examination of P^{32} incorporation may bring about a certain relationship between phosphorus metabolism and the hardness mechanism. In all samples of both acclimation and hardness stages of Bayraklı cultivar, the change of phosphorus content has been related and this relation was getting stronger during development of the hardness and acclimation processes (Figure 3). Consequently, in Bayraklı cultivar, considering the relationship between phosphorus and total metabolism levels, increases in measured biochemical parameters (Figure 1 and 3, ÖNCEL, 1984; Figure 1, 3, 5, 7, ÖNCEL, 1986) showed that the hardness was gained even under the frost; but in the chilled seedling sample of Bayraklı cultivar, it has been found out that the phosphorus content has remained at the same level compared to the control's and that there was no phosphorus loss (Figure 4). Hence, no development related with aging was observed. An unincrease in phosphorus can not only be considered as indication of any relationship, due to lack of details supplied by our limited examination on total phosphorus. Organic and inorganic phosphorus contents, their ratios and p^{32} incorporation need to be examined to draw a final conclusion.

THE CHANGES IN POTASSIUM CONTENT

Potassium is known to be effective on osmotic pressure, permeability, opening and closing of stomata, photosynthesis and growth (VARDAR, 1972), so its increase during the cold acclimation and hardness can be expected. In literature, the results related with potassium and the cold hardness seemed to be obscure. However, it has been explained that the potassium fertilization did not increase freezing tolerance (COOK and DUFF, 1976) but its nutrition increased the cold hardness (LAMB, 1967; GÖKGÖL, 1969; MARSHALL, 1969). Supplying energy for formation of the hardness by various metabolic substances, such as sugar, protein and roles of potassium in the metabolism of these substances, it was considered that there should be an increase in the potassium content for the acclimation and hardness although the results in literature were contradictory. To see if there

was a correlation between the cold acclimation and hardiness, potassium levels were measured and compared with results obtained in the samples against the controls. The relationships between the changes of potassium content and the cold acclimation and hardiness have been examined.

In the frozen unaffected young head sample which is acclimation stage of Samsun top cultivar, potassium increased. In the frozen affected young head sample the potassium content was less than its level in acclimation stage, but it increased as compared with controls. In the frozen affected aged head sample, potassium decreased nearly to control level (Figure 5).

In the acclimation and hardiness samples of the headed Yeşilköy cultivar, on the other hand, potassium decreased (Figure 5).

In the frozen unaffected young head sample corresponding the acclimation stage of the headed Bayraklı cultivar, it was found that potassium content remained at the control level and increased in the frozen affected young and aged head samples which were hardiness stages of this cultivar (Figure 5). During the acclimation and hardening in the samples of Samsun top and Bayraklı cultivars, the increasing of potassium content according to the control may be related with the increase of osmotic pressure and permeability.

In the frozen affected aged head sample of Samsun top cultivar and in the frozen affected young head sample of Bayraklı cultivar, the potassium content which was almost the same as control content can be explained considering that the potassium is a mobile nutrient salt. It is known that the potassium is translocated from old leaves to young ones and it decreases with aging (HEWITT, 1963; VARDAR, 1972). No significant growth during the hardiness namely absence of the growth points to attract the potassium, and accordingly being unchanged of its content are normal.

It was found that the potassium content decreased in the chilled samples of seedling cultivars (Figure 6). This decrease due to the chilling may be related with the decrease of photosynthesis and growth.

When the results of soluble carbohydrate measurements on the headed samples are reviewed, it is seen that these results are contradictory to the ones held on young seedlings if the relation between the potassium distribution and the carbohydrate metabolism are taken

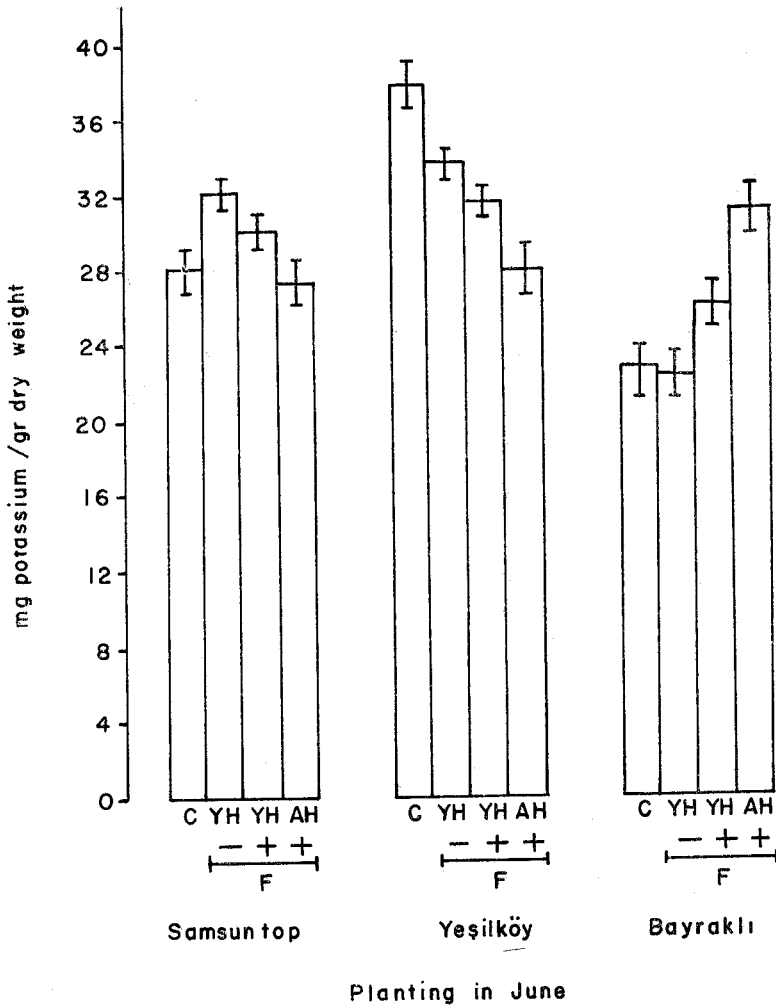


Figure 5. The content of potassium in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

into account. This also shows that the acclimation in the seedlings decreases the permeability, growth and the photosynthesis. In the headed Samsun top and Bayraklı cultivar, the correlation between the soluble carbohydrate and potassium results indicates that the potassium is related with osmotic pressure, and the contents of potassium and soluble carbohydrate are important in adjusting the osmotic pressure of the cell. But in the acclimation stage of Yeşilköy cultivar,

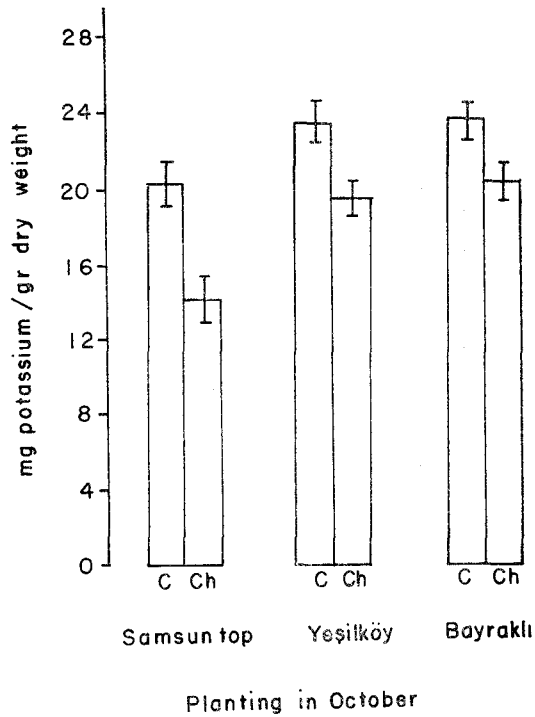


Figure 6. The content of potassium in control and chilled samples of the seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

the potassium content is related with glucose + saccharose and fructose + saccharose levels. In the hardiness stage, glucose + saccharose content decreased while that of fructose + saccharose was increasing. Therefore, the decrease of potassium content in the acclimation and hardiness stages is an indication of the effect of aging.

THE CHANGES IN CALCIUM CONTENT

Although any literatures on the calcium and the cold acclimation and hardiness have not been seen, the high calcium content was found to cause an increase in the low temperature injury (TRESHOW, 1970). In fact, importance of the calcium in the protection of membrane and wall structure and in IAA's activity (POOVAIAH and LEOPOLD, 1976), and the relationship with selective and total permeability show

the role of calcium in the cold acclimation and hardiness. Calcium can not be easily mobilized and it is accumulated in the old leaves rather than those of young ones (VARDAR, 1972). It can not be in a free state and it can not mobile without the decomposition of the structures to which it is bound (RASMUSSEN and et al., 1969; POOVAIAH and LEOPOLD, 1973). Considering these properties of the calcium relevant to its changes during the cold acclimation and hardiness, a relationship between the changes in calcium content and the cold acclimation and hardiness can be seen in the results found in the samples against the controls.

In the frozen unaffected young head sample which was the acclimation stage, and in the frozen affected young and aged head samples which were the hardiness stages of the headed Samsun top cultivar, it was found that the calcium decreased (Figure 7). Decreasing calcium content in the acclimation and hardiness samples of Samsun top cultivar shows that the increase of selective permeability occurs due to the destruction in membranes. Although the calcium decreased against to controls in the frozen affected aged head sample of this cultivar, it showed an increase against to the frozen affected young head sample. These changes may be related to the development of hardiness within this period, senescence took place according to the following facts revealed by POOVAIAH and LEOPOLD (1973). The calcium loss was accompanied and the increase of calcium level by application of its solution retarded the senescence. In their study, they stated that the calcium was removed away from the cell membranes and middle lamellae as a result that RNase activity increase during the development of senescence phenomenon, and consequently, peroxidase activity increased and this enzyme also accelerated the senescence (RUESNIK, 1971; DUYGU, 1976). Therefore, in the head of cabbage, the calcium increase owing to cold effect shows that this increase of calcium protects the head during the senescence of other plant tissues and organs.

Again, in the headed Yeşilköy cultivar, it has been found out that the calcium increased in the frozen unaffected young head sample which was in the acclimation period and decreased in the frozen affected young head sample which was in the hardiness period and increased in the frozen affected aged head sample (Figure 7). In Samsun top and Yeşilköy cultivars in which an increase of calcium has been observed, in the frozen affected aged head samples and in the frozen unaffected young head sample of Yeşilköy cultivar, there was not

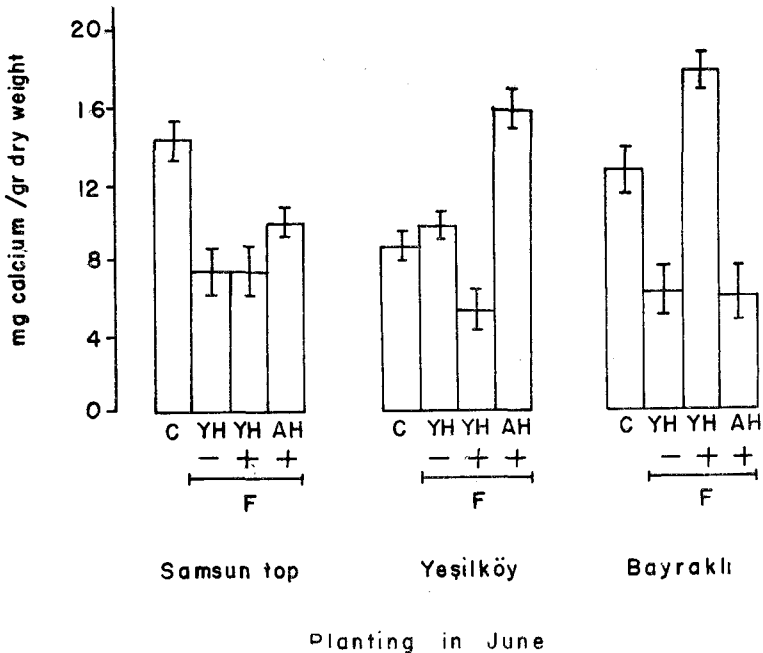


Figure 7. The content of calcium in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

any destruction of membrane and no permeability loss by the effect of senescence and this shows that those samples accumulated calcium to provide hardiness.

It has been found out that the calcium content has decreased only in the frozen unaffected young head sample, that is the acclimation period of the headed Bayraklı cabbage cultivar. While it was increasing in the frozen affected aged head sample during the hardiness period, it decreased in the frozen aged head sample (Figure 7).

In Bayraklı cabbage, the increase of calcium was obtained only in the frozen affected young head sample. In the frozen unaffected young head, and also in the frozen affected aged head samples of Bayraklı cultivar, the decrease of calcium is related with the decomposition of membrane and the loss of selective permeability by senescence. The results relevant to the calcium content show that Yeşilköy cultivar can acclimate by maintaining the selective permeability and can harden. Maintenance of the selective permeability may explain the slow development of acclimation in Bayraklı and Samsun top cultivars and especially the lack of hardiness in Bayraklı cultivar in which a

rapid destruction of the membranes after the freezing period. The membrane destruction rapidly causes senescence, even if the other biochemical hardiness mechanisms work perfectly.

In the chilled seedling samples of cultivars, the changes of calcium content were as follows: The calcium content increased in Samsun top, decreased in Yeşilköy cultivar and remained at the control level in Bayraklı cultivar (Figure 8). This also showed that the loss of selective permeability did not occur in Samsun top, the membrane destruction and the loss of selective permeability occurred in Yeşilköy cultivar and no change of selective permeability in Bayraklı. So, Samsun top cultivar in the seedling stage showed a good hardiness.

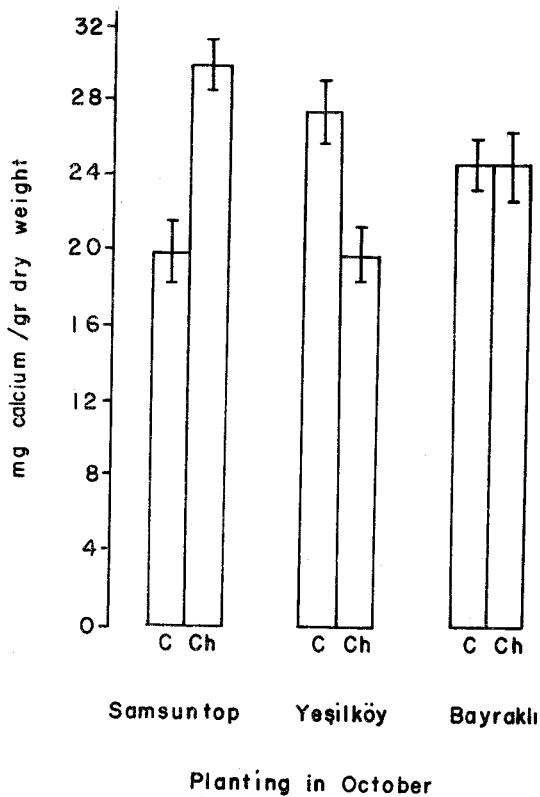


Figure 8. The content of calcium in control and chilled samples of the seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

THE CHANGES IN POTASSIUM/CALCIUM RATIO

While the calcium level in plants is effective on controlling the permeability and growth, potassium is also effective in increasing the

permeability and growth, supplying water to the organs (WOSTMANN, 1942; VARDAR, 1972).

It is known that, the changes in K / Ca ratio causes the parallel changes in physiological activity. Increase in this ratio causes an increase in physiological activity (ABBOTT, 1923; WOSTMANN, 1942). The increase of potassium in our samples, as a matter of fact, decreased the hardness by causing the increase of hydration and selective permeability, swelling of the membranes. Inversely, the decrease in potassium, and the increase of calcium enhanced the hardness, providing dehydration and contraction of membranes. As for the decrease of calcium, it showed that the membranes were decomposed by senescence (DUYGU, 1971). Considering the effects of K / Ca ratio mentioned above, the changes in cold acclimation and hardness, must be evaluated in comparison with controls, and the relationships between the changes of this ratio, the cold acclimation and hardness should be examined.

It was found in Samsun top cultivar that the K / Ca ratio increased in the frozen unaffected young head sample which was in acclimation stage and the frozen affected young and aged head samples, which were hardening stages (Figure 9). Yet, even though the ratio in the hardness samples increased, as compared with the controls; it decreased in comparison to the acclimation stage. This also showed that the increase of selective permeability, in the cold acclimation stage, began to disappear by development of senescence. For, it is known that the senescence causes the membrane destruction and accordingly the loss of selective permeability.

It was found that the K / Ca ratio decreased in the frozen unaffected young head sample corresponding to the acclimation stage, and that it increased in the frozen affected young head sample which was in the hardness stage, and also it decreased in the frozen affected aged head sample of Yeşilköy cultivar (Figure 9). The decrease of K / Ca ratio is related to the senescence in the frozen unaffected young head and the frozen affected aged head samples of Yeşilköy cultivar. As for, the increase of K / Ca ratio, in the frozen affected young head sample, it can be explained considering the high potassium content and the decrease in the calcium, and accordingly the increase selective permeability. That is, the intracellular transport, selective permeability increased in the acclimation. This is also the sign of active metabolism in this stage.

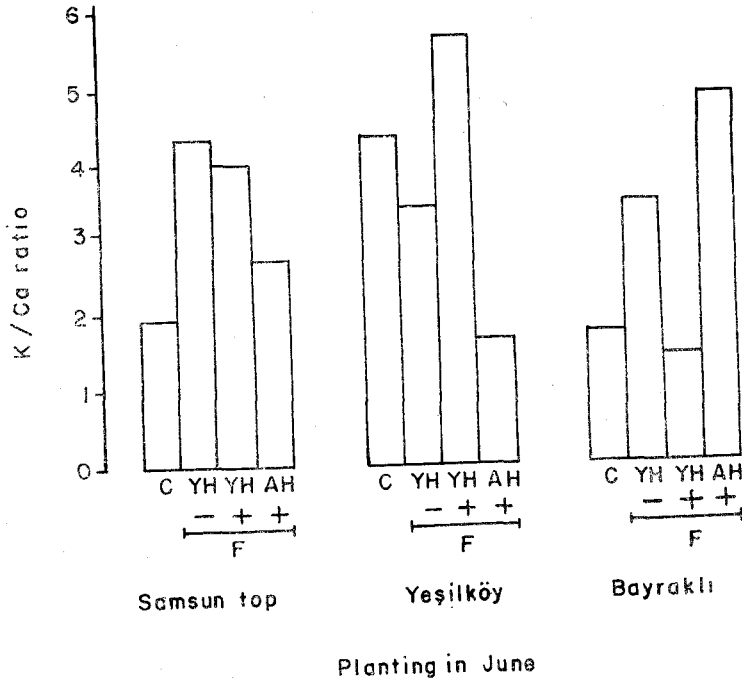


Figure 9. The ratio of potassium/calcium in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

It has been found that the K/Ca ratio increased in the frozen unaffected young head sample which was in the acclimation stage, and decreased in the frozen affected young head sample, which was in the hardiness stage, and subsequently it rose to the highest value in the frozen affected aged head sample of headed Bayraklı cultivar (Figure 9). Probable increase of permeability was indirectly observed only in the frozen affected young head sample. Increasing K/Ca ratio in the frozen unaffected young head and the frozen affected aged head samples is mainly due to the decrease of the calcium and following the destruction of tissues.

The increase of K/Ca ratio is related to the increase of hydration and the selective permeability in the acclimation stage of the headed Samsun top cultivar. Yet, the increase of water content is disadvantageous in the acclimation and hardiness. In hardening samples, the decrease of K/Ca ratio according to the acclimation stage and the increase of calcium content in the frozen affected aged head sample

are the signs of dehydration. For, the calcium increases the hardness causing the membrane to be contracted.

Decreasing the K / Ca ratio in the acclimation stage of the headed Yeşilköy cultivar, and accordingly, increasing the calcium provides the hardness through the membrane contraction and ability of dehydration. The increase of the K / Ca ratio and the decrease of calcium content are related to the destruction of membrane in the hardness stage (frozen affected young head sample). As a result of the calcium content increase, the decrease of the K / Ca ratio is related to the increase of the hardness in the frozen affected aged head sample.

The decrease of calcium because of the senescence, increases the K / Ca ratio in the acclimation stage of the headed Bayraklı cabbage. The decrease of ratio is related to the increase of calcium content in the frozen affected young head sample in the hardness stage. Still, the decrease in the calcium content shows the increase of K / Ca ratio in the frozen affected aged head sample. The rate of increase in the calcium content declines with the effect of the senescence and it can not provide the hardness.

In the chilled seedling samples of cultivars, the K / Ca ratio changes as follows: The ratio decreases in Samsun top and Bayraklı cultivars and increases in Yeşilköy cultivar (Figure 10). As a matter of fact, the increase of the calcium content in Samsun top cultivar, and its being at the same level with control in Bayraklı cultivar and the decrease of it in Yeşilköy cultivar proves this. These also show that the calcium provides the hardness by dehydration and the membrane contraction

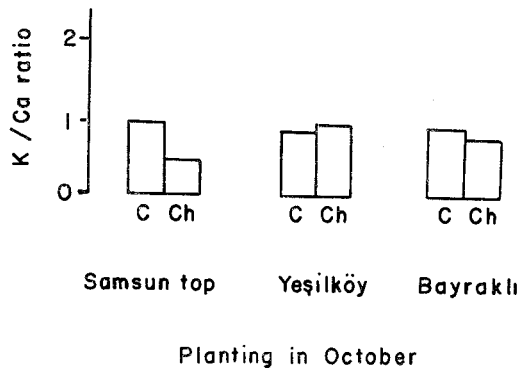


Figure 10. The ratio of potassium / calcium in control and chilled samples of seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

in Samsun top cultivar, and that there is not a loss regarding the calcium, that is, the loss of hardiness and no senescence development in Bayraklı cultivar, but the senescence occurs in Yeşilköy cultivar.

THE CHANGES IN SODIUM CONTENT

Although very few literature related with the sodium nutrient salt and the cold acclimation and hardiness has been obtained, it has been explained that the high sodium content has caused the low temperature injury to increase (TRESHOW, 1970). Since the sodium is a nutrient salt to take the place of the potassium (DUYGU, 1971) and the combinations of calcium, potassium and sodium play an important role in cell hydration, it can be considered that this nutrient salt is related to the cold acclimation and hardiness. During the cold acclimation and hardiness the results related with the sodium content we obtained, having been evaluated according to the controls, the relationships were examined between the changes in sodium content and the cold acclimation and hardiness.

It has been found out that the sodium content has increased in the acclimation and hardiness samples of the headed Samsun top cultivar (Figure 11). Only in the frozen affected aged head sample it has been noticed that the sodium content showing an increase according to the control is less than in the frozen affected young head sample. In this sample the decrease of sodium content can be explained as a result of aging. Consequently, it has been found out that the increases in sodium content were related to acclimation and hardiness of Samsun top.

It has been found out that the sodium content has decreased in the frozen unaffected young head sample corresponding to the acclimation stage but that it has decreased according to the control in the frozen affected aged head sample and that it has increased according to the frozen affected young head sample of Yeşilköy cultivar (Figure 11). While the sodium content decreases in the acclimation stage and in the initial period of hardiness, the increase of it is related to the development of hardiness in the frozen affected aged head sample of Yeşilköy cultivar. This also shows that the formation of hardiness is begun but that it can not be gained.

It has been found out that the increase in the frozen unaffected young head sample corresponding to the acclimation stage and the decrease in frozen affected young head sample which was the hardiness

stage were approximately at the control level and that there is an increase in the frozen affected aged head sample of Bayraklı cultivar (Figure 11). Thus, it has been found out that the increase in the sodium content is related to the acclimation and hardiness of Bayraklı cultivar. The increases in sodium content in samples of each three cultivars show that the plants increase the sodium content to survive under the effect of cold. As a result, it can be said that the sodium content is related to the cold acclimation and hardiness of the headed stages of cultivar (in particular Samsun top and Bayraklı).

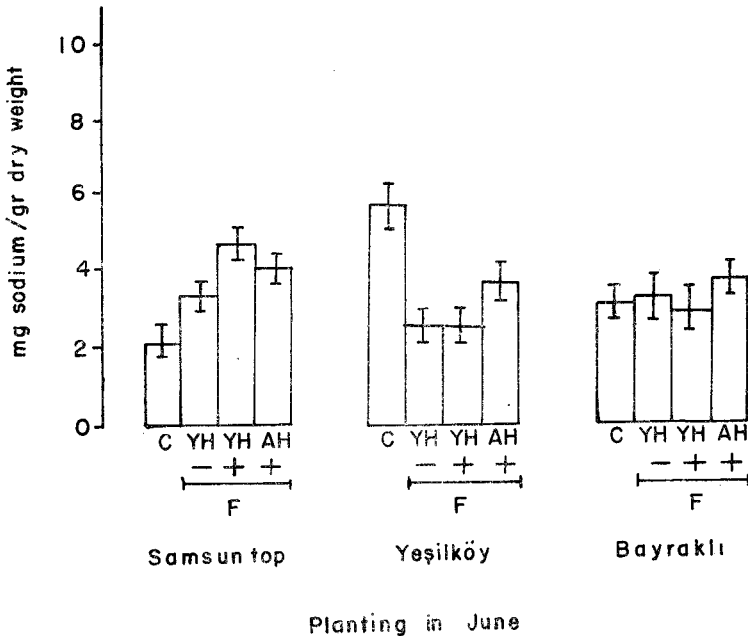


Figure 11. The content of sodium in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

As for, in the chilled seedling samples of cultivars, it has been found out that the sodium content has decreased and that there has been the least decrease in Bayraklı cultivar (Figure 12).

The seedling stage of cultivars is the stage in which growth ability is protected even in cold. For this reason, it can be said that the sodium salt is sent away from the aerial parts of the plant to its root. However,

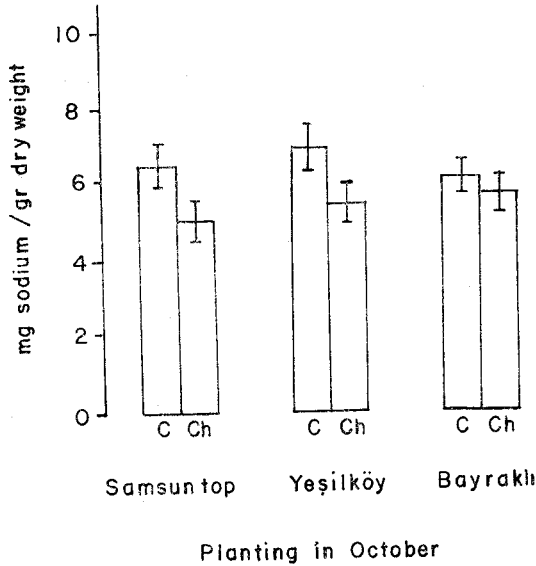


Figure 12. The content of sodium in control and chilled samples of seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

the headed stage of cultivars is the stage in which the plant has completed its growth. The changes obtained in the headed stage are the signs that the sodium nutrient salt is supplied to provide the hardiness.

THE CHANGES IN SODIUM + POTASSIUM / CALCIUM RATIO

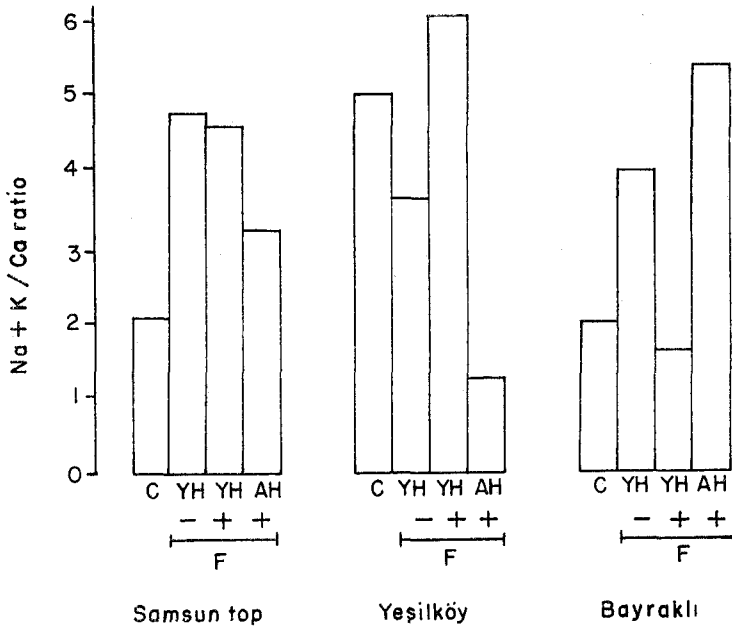
After the changes have been examined in potassium, calcium, K/Ca and sodium contents in the cabbage cultivars used in the study, it is required to examine the effect of these three nutrient salts in the form of $Na + K/Ca$ due to the role of this triplet of sodium, potassium and calcium in cell hydration and the replacement of sodium to calcium and also because the physiological activity increases with the rising ratio of K/Ca and decreases with the diminution of that ratio. Moreover, it is known that the increase of potassium has decreased the hardiness, causing the increase of hydration and selective permeability, swelling of the membranes and that the increase of calcium has provided dehydration, contracting the membranes, and accordingly the hardiness. For this reason, the examination of combined effects of these three nutrient salts has been considered as the ratio of $Na + K/Ca$.

Since the literature related to the features of sodium, potassium and calcium were previously mentioned in the changes related to the sodium and the ratio of K/Ca , they were not repeated here again. During the cold acclimation and hardiness, the changes obtained here, having been evaluated according to the controls, the relationships between the changes in this ratio and the cold acclimation and hardiness have been examined and it has been seen that the changes obtained in $Na + K/Ca$ ratio were similar to the changes obtained in K/Ca ratio. This also shows the importance of $Na + K/Ca$ ratio and of the cations of mono- and divalent such as sodium, potassium and calcium during the cold acclimation and hardiness. $Na + K/Ca$ ratio has been briefly summarized because the changes are given in K/Ca ratio in details.

As seen in Figure 13, the decrease of $Na + K/Ca$ ratio is related to the senescence in the frozen unaffected young head and the frozen affected aged head samples of Yeşilköy and the increase of $Na + K/Ca$ ratio can be explained by the increase of selective permeability, this is also the sign of the increase of active metabolism in the frozen affected young head sample. Consequently, the decrease of $Na + K/Ca$ ratio is related to the increase of calcium, and accordingly the contraction of membrane and dehydration in the acclimation stage of headed Yeşilköy cultivar. The increase of $Na + K/Ca$ ratio together with the decrease of calcium content are related to destruction of membrane in the frozen affected young head sample. The increase of calcium and the decrease of $Na + K/Ca$ ratio show that the hardiness increases in the frozen affected aged head sample.

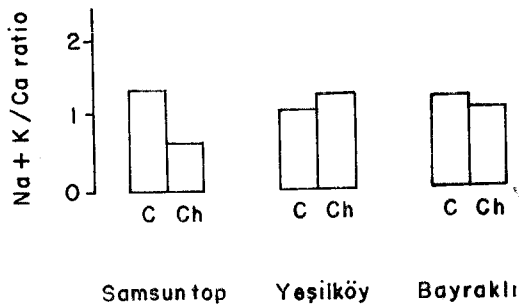
As seen in Figure 13, it has been found out that selective permeability has increased with decreasing of the ratio in frozen affected young head sample of Bayraklı cultivar. As for the increase of $Na + K/Ca$ ratio, it indicates that the calcium decreases, that is, there is a destruction in the tissues of the frozen unaffected young head and the frozen affected aged head samples. Consequently, the senescence causes the calcium to decrease, and accordingly the ratio to increase in the acclimation stage of the headed Bayraklı cultivar. Yet, decreasing of calcium content shows that $Na + K/Ca$ ratio increases in the frozen affected aged sample. This also shows the increase of selective permeability and hydration.

In the chilled seedlings of cultivars, $Na + K/Ca$ ratio changes in the following way: The ratio decreases in Samsun top and Bayraklı cultivars and increases in Yeşilköy cultivar (Figure 14). The calcium



Planting in June

Figure 13. The ratio of sodium + potassium / calcium in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.



Planting in October

Figure 14. The ratio of sodium + potassium / calcium in control and chilled samples of seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

changes that show the senescence occurs in Yeşilköy cultivar and that show there is no senescence and the loss of hardiness in Bayraklı cultivar and that show the hardiness is provided by membrane contraction and dehydration in Samsun top cultivar, exhibits a correlation with the ratio of $\text{Na} + \text{K} / \text{Ca}$. Thus, the relationships between monovalent/divalent ion ratio which is important in permeability and cold acclimation and hardiness have been shown in Samsun top, Yeşilköy and Bayraklı cultivars.

The interrelations obtained between the nutrient salts examined here and seedling and headed stages of cultivars can be summarized as follows.

While the phosphorus shows that headed Samsun top cultivar can acclimate under the cold, and that the hardening capacity is designated but that the hardiness can not be gained, the increase of potassium content can be related to the osmotic pressure and the selective permeability. As for the calcium content, it shows that there is a slow acclimation regarding the selective permeability and the increases in sodium nutrient salt are seen related to the cold acclimation and hardiness of this cultivar.

While the increases of phosphorus are related to the acclimation and hardiness of headed Yeşilköy cultivar, the potassium decreases as a result of aging. The changes in calcium content, however, shows that Yeşilköy cultivar can acclimate and gain the hardiness, and the changes of sodium content show that the hardiness is arisen but that it can not be gained.

While the phosphorus shows that the hardiness is gained, the increase in potassium content is related to the increase of osmotic pressure and selective permeability in headed Bayraklı cultivar. Calcium nutrient salt also shows that the slow acclimation occurs regarding the selective permeability but that the hardiness can not be gained, the increase in the sodium content is also related to the cold acclimation and hardiness of this cultivar.

It has been found out that the contents of sodium and potassium have decreased in the seedling stage of cultivars. The phosphorus nutrient salt also decreases in Samsun top cultivar but the calcium shows that there is no loss of selective permeability. The phosphorus shows that Yeşilköy cultivar can acclimate but that the decrease in calcium content, the destruction of membrane and the loss of selective

permeability occur. Although it has been found out that there is not any loss of phosphorus in Bayraklı cultivar, calcium shows that there is no any change regarding selective permeability. In seedlings, the decrease in the contents of nutrient salts can be the sign of transport from aerial parts of plants to the roots and so, the hardiness can be provided.

DISCUSSION

The results obtained in this research in three cabbage cultivars reveal the relationships between the changes of the nutrient salts such as total nitrogen, phosphorus, potassium, calcium and sodium and the cold acclimation a long with the hardiness.

When the results obtained from the nutrient salts used in evaluations of the cold acclimation and hardiness of the cabbage cultivars are compared to the findings in literature, interpretations can be done as follows:

Except for the acclimation of Samsun top cultivar it has been found that the total nitrogen has decreased both in headed and seedling stages and this shows that the nitrogen is not generally used for hardiness. Our results related to the total nitrogen support LE SAINT (1966) who explained the total nitrogen decreased in cabbage. Furthermore, there are also studies showing that the nitrogen decreases the hardiness (LAMB, 1967) and that the nitrogenous fertilizer should be applied in less the quantities (GÖKGÖL, 1969). As seen above, the small amounts of nitrogen contents for the acclimation and hardiness of cabbage are efficient and they even increase the hardiness according to the results in the literature and ours. When the nitrogenous substance results including the soluble protein, free amino acid nitrogen and total nitrogen are evaluated all together, the results show that they generally decrease during the acclimation and hardiness of cabbage cultivars and that a special nitrogen metabolism necessary for the hardiness is important.

It has been found out that the increases in phosphorus content are related in the acclimation and hardiness of headed Samsun top, Yeşilköy and Bayraklı cultivars and that the phosphorus content has been protected in the seedling stages of Yeşilköy and Bayraklı cultivars. Our results related to the total phosphorus content support the studies (LAMB, 1967; GÖKGÖL, 1969) explaining that the phosphorus nut-

rition generally increases the hardiness. In addition to this, LI, WEISER and VAN HUUSTEE (1966) have found a decrease in inorganic phosphorus during the cold hardiness, but an increase in the total and organic phosphorus. They have explained this situation as an accumulation of the photosynthetic products and the organic phosphorus compounds at low temperatures. The findings of these researchers are consistent with our results. For, the storage of carbohydrate has been observed by us and this has been found to be related to development of the acclimation and hardiness.

Consequently, the increases in the total phosphorus content in the headed stage of cultivars can be generalized for the cold acclimation and hardiness.

It has been found out that the potassium content is related to the acclimation and hardiness in the headed stage of Samsun top and Bayraklı cultivars. Our results related to Samsun top and Bayraklı cultivars support the studies (LAMB, 1967; GÖKGÖL, 1969) explaining that the potassium nutrition increase the cold hardiness in winter crops. On the contrary, in Samsun top and Bayraklı cultivars, it has been found out that the potassium content has decreased in the headed Yeşilköy cultivar and the seedling stage of cultivar. In literature, however, there are also findings pertinent to this view. COOK and DUFF (1976) pointed out that the potassium fertilization did not increase the freezing tolerance of turfgrass crown tissue and JOINER and ELLIS (1964) remarked that the cold injury is not related with the potassium levels in azalea plant. Because the potassium is as important as carbohydrates in regulating the cell osmotic pressure, the parallelism we obtained between the potassium and carbohydrate results show that the potassium can be effective on the accumulation of carbohydrate results in Samsun top and Bayraklı cultivars. COOK and DUFF (1976) examining the changes of carbohydrate and potassium all together, as a result of the potassium fertilization and analysis, found that the potassium content did not have any effect on the accumulation of carbohydrate. Consequently, in our opinion the relationship between potassium and the cold hardiness shows differences in the cultivars according to our results and this can not be generalized.

The variations in calcium content of cabbage cultivars in the cold acclimation and hardiness are quite different. Considering the headed stage, it has been found out that the changes of calcium show a positive correlation in the cold acclimation and hardiness in Yeşilköy cultivar

and that Samsun top and Bayraklı cultivars can acclimate slowly regarding the calcium changes and that there is no good relationship in the period when the hardiness is gained especially in Bayraklı cabbage. It has been observed that there is an increase in the calcium content in seedling Samsun top cultivar and that the calcium content is protected in Bayraklı cultivar.

Literature is inadequate on this subject and it has been stated that the high calcium content has generally increased the low temperature injury (TRESHOW, 1970).

However, since the calcium plays an important role on the wall and membrane structure, it is expected that the calcium is related to the cold acclimation and hardiness, and meanwhile, it can be stated that the calcium changes are important on reflecting the changes in membrane structure. Since, this relationship shows differences according to the cultivars and the physiological stages, anything definite can not yet be stated.

The sodium nutrient salt is as important as potassium in the cold acclimation and hardiness of cabbage cultivars, for the sodium is a nutrient salt taking the place of potassium (DUYGU, 1971). Still, in literature, it has been explained that the high sodium content has generally increased the low temperature injury (TRESHOW, 1970). However, SAKAI and YOSHIDA (1968) have explained that SAKAI (1962) has found an increase in the hardiness of cabbage leaves in NaCl solution. While the increase in sodium content is related to the cold acclimation and hardiness of Samsun top and Bayraklı cultivars, it has been found out that the hardiness is designated but can not be gained regarding the increase of sodium in Yeşilköy cultivar. Consequently, a generalization can not be made.

But in the seedling stage of cultivars, it has been found out that the sodium content has decreased by the effect of cold. This result which is consistent with TRESHOW (1970) shows that the sodium decrease has a role in hardiness in the seedling stage.

When it is generally evaluated, our results show that the nutrient salts are as important in the development and protection of cold acclimation and hardiness as biochemical parameters but that their effects are different in every cultivar. In this subject, however, SAKAI (1962) has designated that these salts have an important effect but it is less than that of glucose in the prevention of the frost injury although the salts such as KCl, NH_4Cl and NaCl have toxic effects.

CONCLUSION

A. Examining the following features, one should take decision so as to obtain suitable results with applications such as fertilization and spraying.

1- It has been observed that the increases in phosphorus content are related to the cold acclimation and hardiness in our study. However, even in literature the same relationship has been observed in winter crops (LAMB, 1967; GÖKGÖL, 1969), in Red-Osier Dogwood (LI, WEISER and VAN HUUSTEE, 1966). For this reason, the increases in phosphorus content can be generalized for cabbage and all plants to provide the cold acclimation and hardiness.

2- A hardiness mechanism to characterize the development of cold acclimation and hardiness only in cabbage; that is, which does not exist in other genus and species, can not be found.

3- In respect to the usefulness degree and timing of application for every cultivar, the parameters to be examined are the increases in sodium, potassium and calcium nutrient salts.

4- Regarding the application, the following can be suggested for the cultivars examined by us:

In the fall, the fertilizers with calcium should be given to Samsun top and Bayraklı cultivars and the fertilizers with sodium and potassium to Yeşilköy cultivar or their solutions should be sprayed in winter.

B. The following suggestions can be made for the improvement studies. These findings show that the cultivars especially storing phosphorus should be selected while selection is made for the production of the new cultivar and other vegetable species which are more probably grown in winter and at least cabbage. Moreover, improvement studies should be done to obtain hardy cultivars and optimal nutritional conditions such as phosphorus, sodium, potassium and calcium should be provided.

C. The following suggestions can be made in respect to growing.

1- In our research, two seed sowing and two seedling planting periods have mainly been considered as of June and October. The changes relevant to mineral salts were generally obtained in the headed samples planted in June. For this reason, plantings can be carried out in June

and even in July and the plants can be left in the field where the lowest temperature is at least approximately -15°C .

2- Bayraklı cabbage cultivar which has a higher cold acclimation and hardiness is suitable for this application.

In conclusion, our research has brought about the necessary fundamental data for the agricultural applications which can be successfully carried out.

REFERENCES

- ABBOTT, O. 1923. In: GARDNER, V.R., F.C. BRADFORD, and H.D. HOOKER, Jr. 1939. The fundamentals of fruit production. McGraw-Hill Book Company, Inc. New York and London. pp. 148-174.
- BEEVERS, L. 1968. In: SPENCER, P.W., and J.S. TITUS. 1972. Biochemical and enzymatic changes in apple leaf tissue. *Plant Physiol.* 49: 746-750.
- COLEMAN, E.A., R.J. BULA and R.L. DAVIS. 1966. Electrophoretic and immunological comparisons of soluble root proteins of *Medicago sativa* L. genotypes in the cold hardened and non-hardened condition. *Plant Physiol.* 41: 1681-1685.
- COOK, T.W., and D.T. DUFF. 1976. Effects of K fertilization on freezing tolerance and carbohydrate content of *Festuca arundinaceae* Schreb, maintained as Turf. *Agron. Jour.* 68: 116-119.
- DROZDOV, S.N., and Z.F. SYCHEVA. 1965. Relationship of the frost resistance of potato haulms with the level of nitrogen metabolism. *Sov. Pl. Physiol.* 12: 274-279.
- DUYGU, E. 1971. Bazı *Vitis vinifera* tarımsal formlarının bir yıllık sürgünlerinde histo ve kemoenolojik bir araştırma Doktora tezi. A.Ü.F.F.
- . 1976. Biochemical and enzymological processes involved in senescence and abscission. *Bitki. Cilt:* 3, Sayı: 1, 80-98.
- GÖKGÖL, M. 1969. Serin iklim hububatı, ziraatı ve ıslahı (buğday, çavdar, arpa ve yulaf). Özyayın Matbaası. İstanbul. pp. 14.
- GUARDIOLA, J.L., and J.L. SUTCLIFFE. 1971. Control of protein hydrolysis in the cotyledons of germinating pea (*Pisum sativum* L.) seeds. *Ann. Bot.* 35: 791-807.
- HAYDEN, R.I., C.A. MOYSE, F.W. CALDER, D.P. CRAWFORD, and D.S. FENSOM. 1969. Electrical impedance studies on potato and alfalfa tissue. *Jour. Exp. Bot.* 20: 177-200.
- HEWITT, E.J. 1963. The essential nutrient elements: Requirements and interactions in plants. In: STEWARD, F.C. 1963. *Plant Physiol. A Treatise.* Academic Press. New York. Vol. 111: 143-149.
- HOWELL, G.S., and G.A. JUNG. 1965. Cold resistance of Potomac Orchardgrass as related to cutting management, nitrogen fertilization and mineral levels in the plant sap. *Agron. Jour.* 57: 525-529.

- JOINER, J.N., and E.R. ELLIS, JR. 1964. Effects of varying levels of nitrogen and potassium on cold hardiness of "George Tabor" Azaleas. Proc. Fla. St. Hort. Soc. 77: 525-527.
- KACAR, B. 1972a. Nitrik-perklorik asit karışımı ile yağ yakma. In: Bitki ve toprağın kimyasal analizleri: II. Bitki analizleri. Ankara Üniversitesi Basımevi. Ankara. pp. 47-49.
- . 1972b. Organik azot tayin metodları. Metod I. In: Bitki ve toprağın kimyasal analizleri: II. Bitki analizleri. Ankara Üniversitesi Basımevi. Ankara. pp. 59-61.
- . 1972c. Fleymfotometrik metodlar. Bitki eriyiklerinde sodyum tayini. In: Bitki ve toprağın kimyasal analizleri: II. Bitki analizleri. Ankara Üniversitesi Basımevi. Ankara. pp. 146-149.
- . 1972d. Fleymfotometrik metodlar. Bitki külli eriyiklerinde potasyum tayini. In: Bitki ve toprağın kimyasal analizleri: II. Bitki analizleri. Ankara Üniversitesi Basımevi. Ankara. pp. 117-120.
- KAWANA, A., M. NAKAHARA, B. SUGIMATO, and H. HURUHATA. 1964. In: ALDEN, J., and R.K. HERMANN. 1971. Aspects of the cold-hardiness mechanism in plants. Bctan. Rev. Vol. 37: 37-116.
- LAMB, C.A. 1967. Physiology. In: QUINSENBERRY, K.S., and L.P. REITZ. 1967. Wheat and wheat improvement. Amer. Soc. Agron. pp. 181-223.
- LE SAINT, A.M. 1966. In: LEVITT, J. 1972. Responses of plants to environmental stresses. Academic Press. New Ycrk. pp. 132.
- LI, P. H., C.J. WEISER, and R. VAN HUYSTEE. 1966. The relation of cold resistance to the status of phosphorus and certain metabolites in Red-Osier Dogwood (*Cornus stolonifera* Michx.). Plant and Cell Physiol. 7: 475-485.
- LYNCH, J., and S. GOLDWEBER. 1956. In: JOINER, J.N., and E.R. ELLIS, JR. 1964. Effects of varying levels of nitrogen and potassium on cold hardiness of "George Tabor" Azaleas. Proc. Fla. St. Hort. Soc. 77: 525-527.
- MARSHALL, H.G. 1969. Effect of seed source and seedling age on the freezing resistance of winter oats. Crop Sci. 9: 202-205.
- ÖNCEL, I. 1984. A study on the relation of cold acclimation and hardiness to mineral nutritional and biochemical fluctuations of three agricultural forms of *Brassica oleracea* L. I-Soluble carbohydrate and starch fluctuations. Commun. Fac. Sci. Univ. Ankara. Série C, 2 (2): 13-37.
- . 1986. A study on the relation of cold acclimation and hardiness to mineral nutritional and biochemical fluctuations of three agricultural forms of *Brassica oleracea* L. II-Total RNA and DNA, free amino acid nitrogen and soluble protein fluctuations. Commun. Fac. Sci. Univ. Ankara. Série C. V. 4: 81-103.
- POOVAIAH, B.W., and A.C. LEOPOLD. 1973. Inhibition of abscission by calcium. Plant Physiol. 51: 848-851.
- , and ———, 1976. Effects of inorganic solutes on the binding of auxin. Plant Physiol. 58: 783-785.

- RASMUSSEN, H.P., and M.J. BUKOVAC. 1969. A histochemical study of abscission. *Amer. Jour. Bot.* 56: 69-76.
- RUESNIK, A.W. 1971. The plasma membrane of *Avena* coleoptile protoplast. *Plant Physiol.* 47: 192-195.
- SAKAI, A. 1960. Relation of sugar content to frost hardiness in plants. *Nature.* 185: 689.
- , 1962. Studies on the frost-hardiness of woody plants. I-The causal relation between sugar content and frost hardiness. *Cont. Inst. Low. Temp. Sci. Series B.* 11: 3-40.
- , and S. YOSHIDA. 1968. The role of sugar and related compounds in variations of freezing resistance. *Cryobiology.* 5: 160-174.
- TRESHOW, M. 1970. Environment and plant response. McGraw-Hill Book Company. New York, pp. 77-94.
- VARDAR, Y. 1972. Bitki Fizyolojisi Dersleri. I-Bitkilerin metabolik olayları. Ege Üniversitesi Matbaası. İzmir.
- WOSTMANN, E. 1942. In: BIEBL., R. 1958. Der Einfluss der Mineralstoffe auf die Transpiration. In: RUHLAND, W. 1958. *Encyclopedia of Plant Physiol.* Springer-Verlag, Berlin. Vol. IV: 382-426.