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# 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

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ABSTRACT

The relation of cold acclimation and hardiness with total RNA and DNA, free amino acid nitrogen and soluble protein in various plants has long been known. In this study, this relation has been investigated on non-dormant cabbage. The agricultural aim of our study is to establish the hardy cabbage cultivar, to evaluate the biochemical parameters to be used in improvement studies and to obtain the yield in winter with the applications to increase the hardiness.

The effect of cold on three agricultural forms of white head cabbage has been 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 freeze, chilled only.

The increases of total RNA and DNA contents during the cold acclimation and hardiness have been accepted as related with this fact. The increases of total RNA and DNA contents in the headed samples of cultivars are related to cold acclimation and hardiness. Except for the chilled samples of one cultivar, there is a relationship with the content of total RNA in the chilled samples of two cultivars. There is no relationship with the content of total DNA in the chilled samples of seedlings of three cultivars. It has been found out that the changes of free amino acid content showed differences in the cultivars. As for in the headed cultivar, the increase in the free amino acid content is related to cold acclimation and hardiness stages being different than the other cultivars. The free amino acid nitrogen has decreased in the samples of chilled seedlings of the cultivars. It has been shown that the soluble protein content decreased in the seedling samples of cultivars and in the head samples of one cultivar. In the headed cultivar, the increase of soluble protein content is related with

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cold hardiness stage and in the other headed cultivar it is related with cold acclimation and hardiness.

The results showed that nucleic acids, soluble proteins and free amino acids which are fundamental metabolic substances of plants were as important in the development of of cold acclimation and hardiness as carbohydrates.

## INTRODUCTION

The literature related with soluble protein, total RNA and DNA and free amino acid nitrogen in cold acclimation and hardiness was presented in the previous paper (Öncel, 1984).

It was determined by various researchers Sakai and Yoshida (1968), Shomer-Ilan and Waisel (1975) that soluble, enzyme proteins were related to cold hardiness and it was also determined by Gusta and Weiser (1972) that in cold acclimation the increases in the synthesis RNA and protein played an important role in hardiness and it was shown that in the cold hardiness the contents of RNA, DNA and soluble protein in hardy herbaceous plant species might be greater than those in tender herbaceous plant species (Jung, Shih and Shelton, 1967a). The relationship of nucleic acids with cold hardiness has studied because of their importance in protein synthesis.

As proteins were produced by polimerization of amino acids, the relationship of amino acids with cold hardiness was studied. Although Li, Weiser and Van Huystee (1966) have shown that proteins increase in parallel to the decreasing of free amino acids in woody plants during the cold hardening periods. It has been found out that relative increases in the contents of soluble protein and amino acid in the hardy species of herbaceous plants during the cold hardening period were much higher (Pauli and Zech, 1964). Thus it has been shown that the changes in RNA, DNA and amino acids and consequently protein metabolism were responsible for the development of hardiness in woody and herbaceous plants.

Considering that cold acclimation and hardiness may have been resulted from the interaction among various biochemical factors, the aim of our study is to examine biochemical mechanism containing soluble protein, total RNA and DNA and free amino acid nitrogen in cabbage which is characterized by having a non-dormant stage differing woody plants and to evaluate all together.

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

## MATERIAL AND METHODS

Being prepared of the material (sowing the seeds, planting the seedlings and preparing the soil), sampling method, taking the samples and their preparation for analysis were presented in the previous paper (Öncel, 1984).

### Methods

#### Total RNA and DNA Determination

The Holdgate and Goodwin method (1965) was used for the extraction of total RNA and DNA. The aliquots of total RNA and DNA were determined by colorimetric method using diphenylamine reagent according to Pederson (1969).

#### Free Amino Acid Nitrogen Determination

The Hedley and Stoddart method (1972) was used for the extraction of free amino acid nitrogen. The protein was precipitated in the aliquots and supernatant of free amino acid nitrogen was determined with an ammonium electrode by an ionanalyzer.

#### Soluble Protein Determination

Samples were homogenated with tris – HCl and then following centrifugation at 10.000 x G, the supernatant was completed to a known volume according to Hedley and Stoddart (1972). The aliquots of soluble protein were determined by colorimetric method using biure reagent according to Gornall, Bardawill and David (1949).

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

## RESULTS

### Sampling

The sampling method was presented in the previous paper (Öncel, 1984).

## The Changes in the Content of Total RNA and DNA

There are increases in the contents of total RNA and DNA during the period of cold acclimation and hardiness (Jung, Shih and Shelton, 1967a). When compared to the control, the results related to the contents of total RNA and DNA during the period of cold acclimation and hardiness have shown that there is a relationship between the increases of total RNA, DNA and cold acclimation and hardiness.

The patterns of changes of both parameters in headed Yeşilköy cultivar are similar to each other during the period of cold acclimation and hardiness (Figure 1 and 3). In the frozen unaffected young head cabbage which was a sample of acclimation, it was found that the contents of total RNA and DNA were related to the acclimation (Figure 1 and 3). They increased during the period of hardiness in the samples of frozen affected young head and frozen affected aged head when compared with the controls but a decrease was apparent if compared with the developments in acclimation period (Figure 1 and 3). This was the sign of aging, as it is known since Sacher (1973) showed that RNA and DNA decreased by hydrolysis during aging and senescence.

The changes of both parameters in the headed Bayraklı cultivar are similar to each other during the period of cold acclimation and hardiness (Figure 1 and 3). In the frozen unaffected young head cabbage, a sample of acclimation, it has been found out that the changes are related to the acclimation (Figure 1 and 3).

The behaviour differences have been observed in the samples of frozen affected young head, frozen affected aged head of Bayraklı cabbage cultivar, namely in the hardiness stage. Although there is an increase in the frozen affected young head samples when compared to the control, there is a decrease in spite of the acclimation stage. The resultant decreases in affected samples may be caused by hardening and aging which develop at the same time but with some difference in their rates. Yet the increase in the frozen affected aged head stage of this cultivar is more than that in the acclimation stage. This increase has been gained by the increase of hardening ability (Figure 1 and 3).

The content changes of total RNA and DNA of the headed Samsun top samples are similar to each other during the period of cold

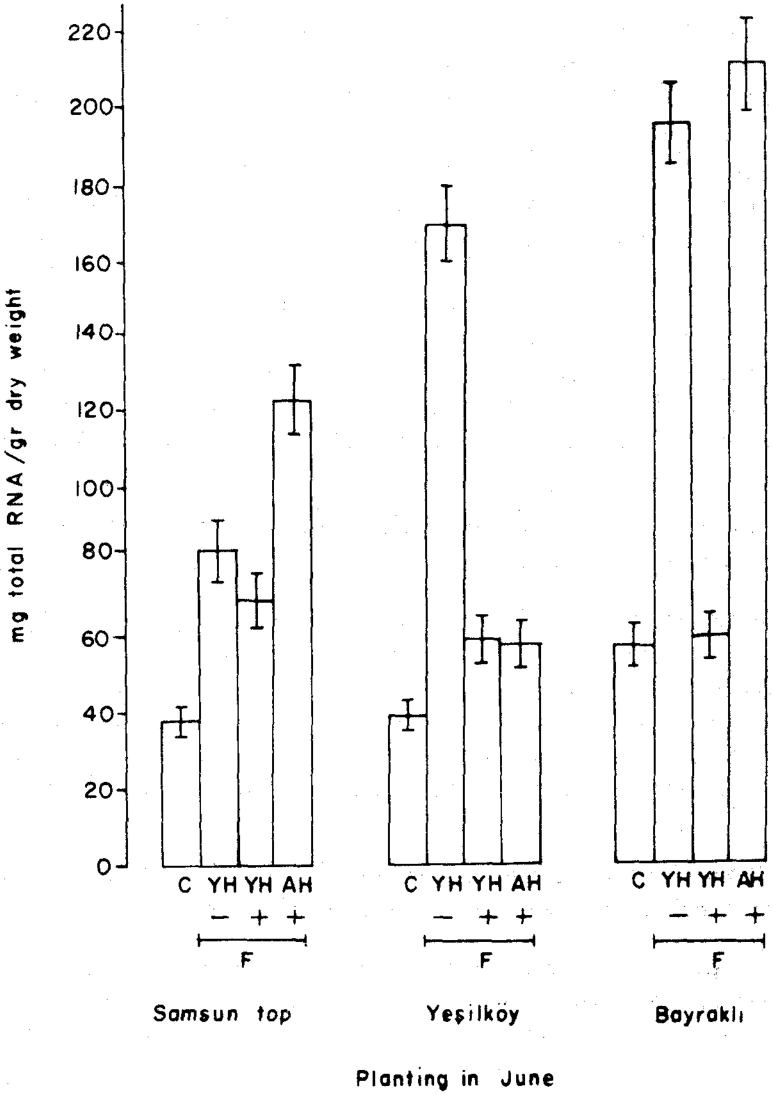


Figure 1. The content of total RNA in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

C: Control — F: Frozen
YH: Young head — —: Unaffected
AH: Aged head — +: Affected

acclimation and hardiness (Figure 1 and 3). In the frozen unaffected young head cabbage, a sample of acclimation, the changes of total RNA and DNA contents are related to cold acclimation. While the content of total RNA increases in the frozen affected young head sample, as a result of being affected in the hardiness stage when compared to the control, it decreases in accordance with the acclimation period. This result can be explained as the effect of aging, that is, there is insufficient hardiness. Yet the increase of RNA in the frozen affected aged head sample in accordance with the acclimation stage may be considered as the increase of hardening ability (Figure 1). It has been found out that the hardiness is related to the content of DNA in the frozen affected young head and frozen affected aged head samples, which correspond the hardening stage of Samsun top cultivar. Hardening process in this cultivar is parallel to the increase of DNA (Figure 3).

The contents of RNA and DNA of the seedling forms of cultivars have changed as described below:

Although there is not any relationship with the content of total RNA in the chilled samples of Samsun top and Bayraklı cabbage cultivars, there is a relationship in the chilled samples of Yeşilköy cultivar (Figure 2). It has been shown that there is a parallelism between total RNA in unaffected young head sample in acclimation stage and that of chilled sample in seedling acclimation stage of Yeşilköy cultivar (Figure 1 and 2).

There is no similar relationship in the chilled samples of seedlings of three cultivars in their total DNA levels (Figure 4).

It has been observed that the behaviours of young seedlings and headed cabbages were different in their RNA and DNA levels. What it is essential in the headed cabbages is the value obtained from the frozen unaffected young head cabbage, which is the acclimation sample. When the results are compared with the ones held in controls for the affected samples which were losing their liveliness, the changes were related to acclimation, hardiness and aging developed at the same time. Moreover, the cultivars showing an increase in affected samples may be taken as forming hardiness, for it is difficult to think of another synthesis programme. For this reason, it would be better to examine the later stages of samples affected by frost. Again, physical differences of plant should be taken into consideration. The dif-

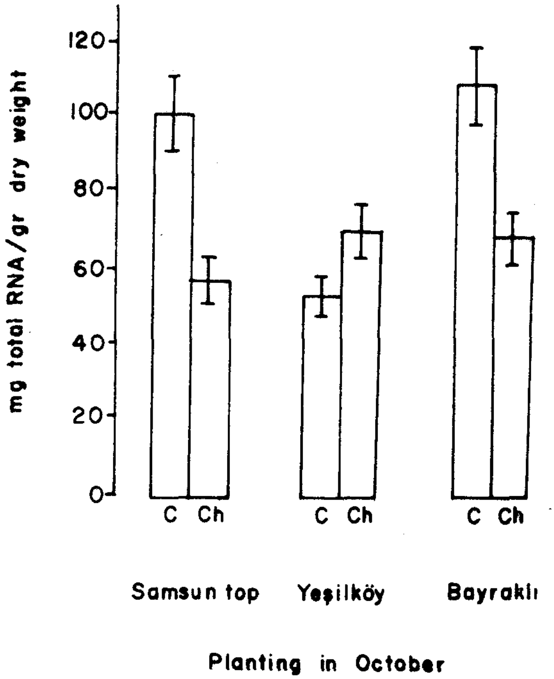


Figure 2. The content of total RNA in control and chilled samples of the seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

ference of formation rate of cabbage leaves leads to the difference of hardening rate of cultivars, even though biochemical hardening has not developed yet. Cultivars with the leaves closely overlapped lose less heat and they are less affected. But the young seedlings have growth points and they increase the hardiness by different mechanisims, such as active growth, rapid metabolism and high osmotic pressure (Sakai and Yoshida, 1968).

In the headed cabbages, the increase of total DNA may be the sign of facts such as the increase in the number of mitochondria, and consequently the rise of respiration capacity, the increase of metabolic DNA and the rise of synthetic capaticty of new proteins related with hardiness.

## The Changes in the Content of Free Amino Acid Nitrogen

It has been found that free amino acid nitrogen decreased in the frozen unaffected young head samples which correspond in acclima-

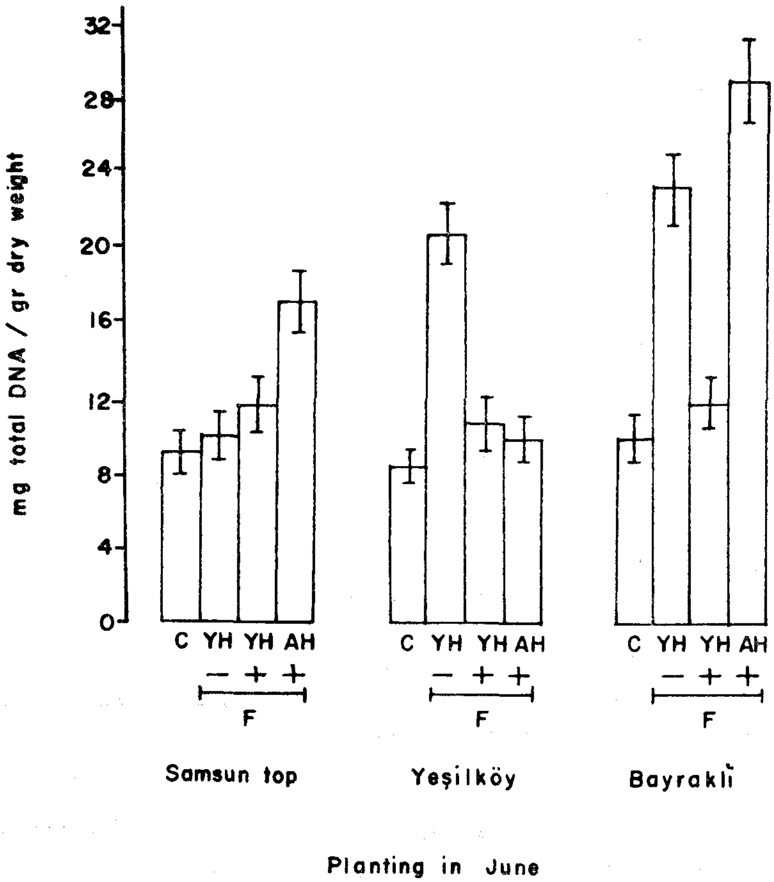


Figure 3. The content of total DNA in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

mation stages of headed Samsun top and Yeşilköy cultivars (Figure 5). But it increased as a result of being affected in the frozen affected and hardening young heads of Yeşilköy cultivar. It decreased in the frozen affected aged head sample as a result of aging (Figure 5). In the frozen affected and hardening young head sample of Samsun top cultivar, its level was higher than the sample in acclimation period. But when compared to the control, it seemed decreased. Similarly, it has been found that the content of free amino acid has decreased also in the frozen affected aged head sample (Figure 5). There is a decrease in the content of free amino acid in the Samsun top and Yeşilköy cultivars. Yet in the headed Bayraklı cultivar, we have observed that there was

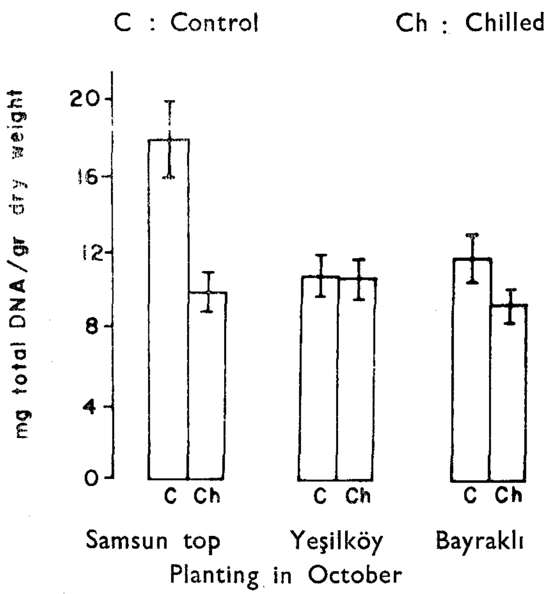


Figure 4. The content of total DNA in control and chilled samples of the seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

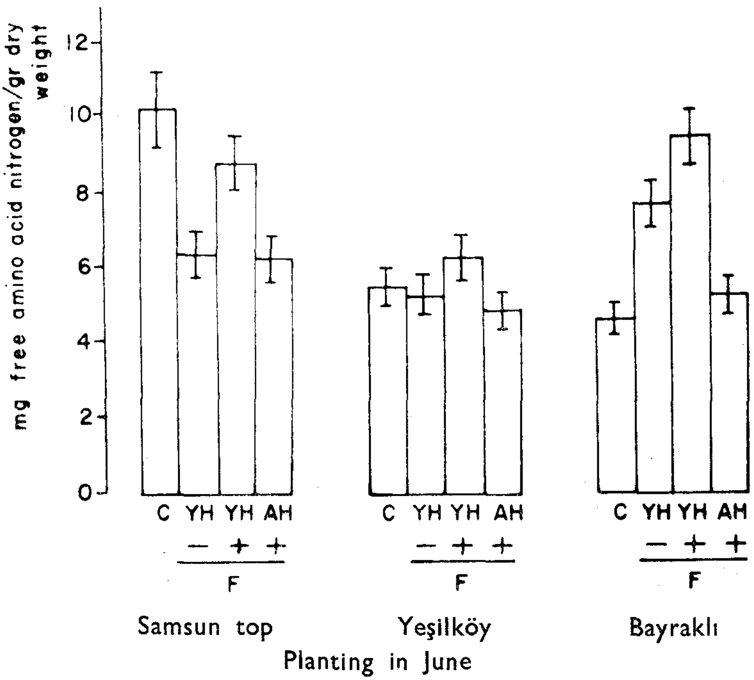


**Figure** 5. The content of free amino acid nitrogen in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

a relationship between the increase in free amino acid nitrogen being different from the Samsun top and Yeşilköy cultivars in cold acclimation and hardiness periods. In the acclimation period of this hardy cultivar, the frozen unaffected young head sample and in the frozen affected and hardening young head sample nitrogen was found higher but it decreased in frozen, affected aged head sample (Figure 5).

Nitrogen decreased in the samples of chilled seedlings of all of the three cultivars (Figure 6). Moreover, a decrease of nitrogen showed parallelism in the acclimation samples which were either headed or seedling forms of Samsun top and Yeşilköy cultivars (Figure 5 and 6).

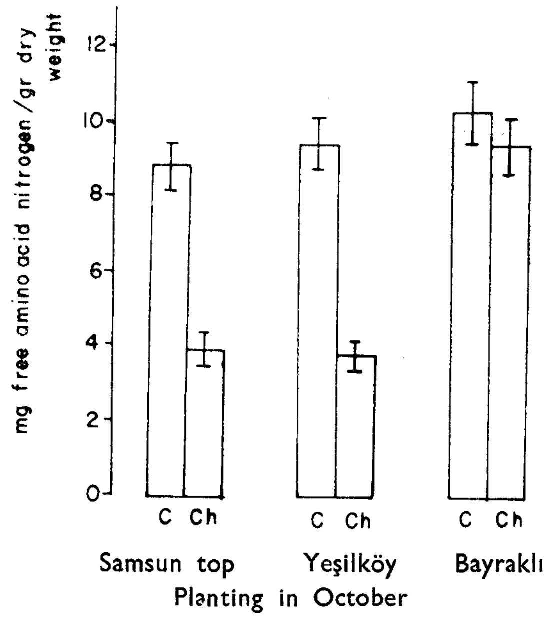


Figure 6. The content of free amino acid nitrogen in control and chilled samples of the seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

The changes in the content of free amino acid nitrogen have been found to show a decrease by some investigators (Li, Weiser and Van Huystee, 1966) but an increase (Pauli and Zech, 1964) during the cold acclimation and hardiness period was also reported (Le Saint, 1966). He related the decrease with the hardiness development in cabbage, and concluded that the decrease provided hardiness development during the period of cold acclimation and hardiness. As it is seen, there are contradictory findings regarding the relationship between the development of acclimation-hardiness and amino acid metabolism. As a matter of fact, during the period of acclimation and hardiness an increase may be expected in amino acids, because the high levels of soluble proteins are necessary for the hardiness development (Pauli and Zech, 1964).

Increasing free amino acid nitrogen in headed Bayraklı cultivar was taken as the indication of the increase of a special amino acid content in the plant body by affecting the total content as cited first by Li, Weiser and Van Huystee (1965). Acclimation and hardiness development in cabbage cultivars resulted both an increase and decrease in this nitrogen fraction shows the requirement to examine not the total amino acid level but the changes in amino acid composition. Amino acids which are one of the fundamental metabolic substances of a plant are expected to be consumed in acclimation and hardiness development of cabbage which grows in cold and does not have a dormant period. So in cabbage cultivars, aging should be taken into consideration. It is a known fact that the content of amino acid increases with aging of leaves (Plaisted, 1958; Beevers, 1968). This has been observed in the headed Bayraklı cultivar. But, on the contrary, there are also studies which show that leaves lose amino acids, but plant roots and stem do not accumulate them until first frosty periods (Lewington, Talbot and Simon, 1967). As it is seen, in literature, the relationship between amino acid metabolism and aging is also observed. For this reason, considering the behaviour of individual amino acids between cold acclimation and hardiness and examining the changes relevant to their synthesis and hydrolysis will reveal whether there is the necessity for amino acid to increase or decrease.

Our findings do not have enough qualities to make clear whether amino acids are necessary for cold acclimation and hardiness or not.

## The Changes in the Content of Soluble Protein

It has been explained that there is generally an increase in the content of soluble protein during the period of cold acclimation and hardiness (Siminovitch and Briggs, 1949, Gusta and Weiser, 1972).

There are enzymes in the soluble protein phase and the increase in the content of the soluble protein indicates the active metabolism increase. During the period of cold acclimation and hardiness the increase in active metabolism provides the cell's vital activities to proceed in a certain direction (Gerloff, Stahmann and Smith, 1967). Moreover, it is known that there is also an increase in the content of soluble protein with aging and senescence by the effect of cold (Beevers, 1968). In addition to this, in cold the decrease of soluble proteins is normal as a result of denaturation according to the control and this decrease is the result of frost effect (Levitt, 1972). As for the increase of the latter, it should be related to the synthesis of new enzymes necessary for acclimation and hardiness. As a matter of fact, there is evidence which shows that metabolism has been directed to a specific purpose in this way and it has been observed that protein synthesis rate increased while soluble protein decreased during the aging and senescence (Addicott, 1968). But new synthesis rate was not fast enugh to compansate the loss of protein in senescence. Taking all these facts into consideration, the changes of protein in our cultivars can be summarized as follows.

Although there is very little increase in the frozen unaffected young head sample of headed Yeşilköy cultivar, which corresponds in the acclimation stage, in the frozen affected young head sample, there is a decrease as a result of being affected in the hardening stage. But, the increase in the frozen affected aged head which was so little was almost at the level of control. It was less than that taken from the plants in acclimation stage (Figure 7).

In the acclimation and hardiness stages of the headed Samsun top cultivar, it has been found that soluble protein content decreased (Figure 7). The soluble protein content decreased in the frozen unaffected young head corresponding the acclimation period of Bayraklı cultivar. As a result of being affected, the soluble protein content increased in both samples: Frozen affected young head and frozen affected aged head, but this increase was not high enough to reach to the control level (Figure 7). This increase in frozen affected aged head sample of Bayraklı cultivar was more than that found in the frozen affected young head sample of this cultivar. This increase is related to acclimation and hardiness and it shows that the plant increased its soluble protein snythesis rate in order to provide its hardiness. Yet this increase is small due to senescence development and also to physi-

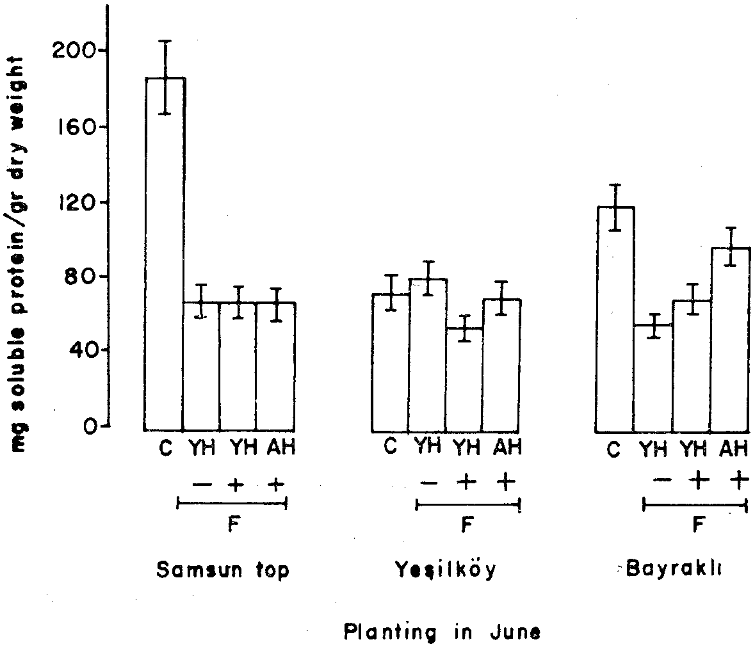


Figure 7. The content of soluble protein in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

cal breaking of membranes down. The same result has also been obtained in the frozen affected aged head sample of Yeşilköy cultivar.

It was found that content of soluble protein decreased in the samples of chilled seedlings of the cultivars (Figure 8). The decreases in the soluble protein content in seedling forms of Bayraklı and Samsun top cultivars were less than that found in Yeşilköy cultivar.

In the head samples of Samsun top cultivar, in the frozen affected young head sample of Yeşilköy and in the frozen unaffected young head sample of Bayraklı and also in the samples of chilled seedlings of these cultivars, the decrease in soluble protein was the indication of frost effect and aging. Le Saint (1966) has shown that the decrease in the protein nitrogen was concomitant with the increase in hardiness development in cabbage. Harper and Paulsen (1967) also found that soluble protein decreased while the hardening of "Pawnee' wheat crown tissue was being formed. But when evaluating,

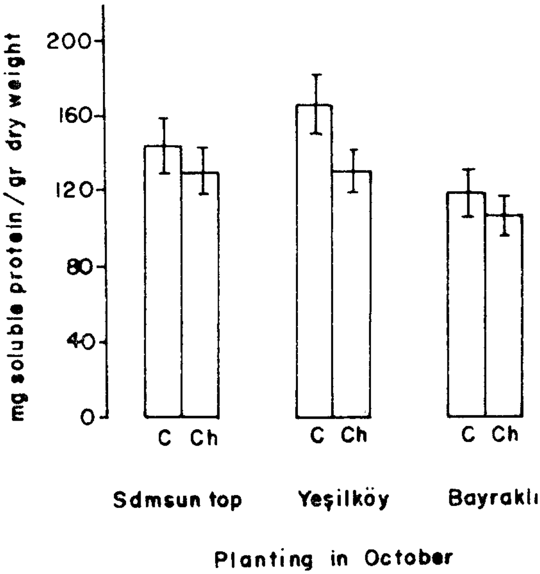


Figure 8. The content of soluble protein in control and chilled samples of the seedings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

it should be taken into consideration that the cabbage is a growing plant in winter, especially in acclimation period and being different from the other plants in that respect. Growth can also lead to the fall of concentration, by diluting soluble protein. As a matter of fact, referring this, Levitt (1972) put forward that freezing tolerance develoment was proportional to the growth rate, and for this reason, accumulation of substances such as soluble proteins in cabbage could not be expected. Actually, only the measurement of soluble protein content does not clearly show the relationship between this parameter and hardiness. But examination of protein synthesis rate, proteolitic activity and soluble protein content changes in the same samples can lead to the right target comparatively. As a matter of fact, in cabbage, Morton (1969) measured protein synthesis rate and soluble protein content to examine the relationship between hardiness and the the sulphydryl content in proteins, and he also found an increase in soluble protein fraction. We also observed this increase in the samples taken from later periods of controls. But in our results, the decrease of soluble protein content in the seedling samples of cultivars and in the head samples of Samsun top cultivar, comparatively with the controls, may be due to the higher rate of decrease of dry and fresh weight, or can be related to the direct effect of freezing. In conclusion, during the acclimation and hardiness period of the headed Yeşilköy cultivar and during the hardiness period of Bayraklı cultivar, the increases in soluble protein are related to cold acclimation and hardiness.

It has been found out that total RNA and DNA contents increased during cold acclimation and hardiness of cabbage cultivars. Increasing DNA may be the sign of the cell growth, and accordingly the potential cell division. Among the cultivars, the highest DNA increase has been found in the hardy Bayraklı cultivar. This increase may be indirectly related to respiration increase as the result of the mitochondri number and mitochondrial DNA. The parallel increase in RNA implies the increase of protein synthesis rate. Increasing protein content is related to the growth, and accordingly, to the increase of the structural protein content and also the enzyme proteins, soluble proteins. Our findings have shown that in the head samples of Yeşilköy and Bayraklı cultivars, there is a correlation between soluble protein content and cold acclimation and hardiness, for the increase in the soluble protein content is related to the acclimation and hardiness. The increase and decrease patterns of soluble protein in the cultivars and their different stages may show that protein is synthesyzed, but decomposed during the hardiness stage; Parallel RNA increases may not be related to the level of soluble proteins, but to the increase of protein turnover rate. This hypothesis can explain the development of closely related acclimation and hardiness developments. In cold periods, proteins and enzymes which are the elements of steady development of hardiness are synthesyzed and that the older ones are decomposed (Cox and Levitt, 1969). They stated, depending on their findings, that total nitrogen consisting of free amino acids, so-soluble proteins and structural proteins, was not increasing, while soluble proteins increasing and amino acids decreasing. Then the amino acids available were to be used in the synthesis of enzyme proteins. As a matter of fact, the results have been obtained in this study. In the frozen unaffected young head sample, which is acclimation stage of Yeşilköy cultivar amino acid content increased and protein content was found to be stable. This can be explained by accepting that the amino acids were coming from roots. This picture has been obtained in Bayraklı cultivar. In the frozen affected young head sample of Samsun top cultivar, it has been found out that the amino acids increased, while soluble protein was stable but this increase has not reached to the control level. This has also shown that there was some amino acid translocation, even if it was very little.

While the relationships have these features in the headed samples of the cultivars, an increase in the RNA content has been obtained only in Yeşilköy seedlings. Decreasing of nitrogenous compounds in head stage and seedling stages of cultivars generally, means that they are consumed to provide the hardiness. This can also be related to increased levels of carbohydrates for hardiness. In our study, it has been observed that there are increases of carbohydrates in the acclimation and hardiness periods. In the stage where there is no available photosynthesis or where the photosynthesis is very low, carbon chains may be used in the carbohydrate synthesis when the nitrogenous compounds are being broken up. Moreover, as mentioned before, this may be related to the specification of metabolism for hardiness, that is, the proteins which play no roles in hardiness were to be left out (Wollgiehn, 1967).

## DISCUSSION

The results obtained in this study on the three cabbage cultivars used reveals the relationships of the biochemical parameters such as total RNA and DNA, free amino acid nitrogen and soluble protein with the cold acclimation and hardiness. When the biochemical parameter measurements used in this study of the cold acclimation and hardiness of cabbage cultivars are compared to the findings in literature, some results can be held as follows.

We have found that there was a relationship between cold acclimation and hardiness in the headed samples of the cabbage cultivars and the content of total RNA and DNA. In the Bayraklı cabbage, known as hardy, the total RNA and DNA contents are higher when compared to the other cultivars. These results support the findings obtained by Shih, Jung and Shelton (1967). The increase of total RNA and DNA was parallel to cold hardiness increase in plants, and that RNA and DNA contents were higher in the hardy alfalfas than those in non-hardy ones. In addition to these, it has also been found here that, total RNA contents were higher than total DNA in cabbage cultivars. In 1967 Shih, Jung and Shelton in alfalfa, and in 1969 Li and Weiser in apple shoots pointed that total RNA content was higher than the DNA content. Yet Jung, Shih and Shelton (1967a) found that DNA content was higher than RNA, in alfalfa and during the hardening period of pseudoacacia, without any change in the content of DNA, there was an increase in RNA content. Their findings are in contrary to our findings relevant to the DNA content (Siminovitch, 1963). The relationships between hardening and total RNA and DNA contents were obtained in headed stages of cultivars and in the seedling stage of only the Yeşilköy cultivar, it has been found that there was a total RNA increase. In the chilled seedling samples of the cultivars there was no relation with total RNA and DNA content, this can be explained as our findings on the changes of mineral nutrients in these material.

As a matter of fact, it is known that the decline in phosphorus levels causes DNA and RNA synthesis rates to decrease (Hewitt, 1963). In seedlings during the cold acclimation stage, a decrease in the content of phosphorus or its unchanged content can be taken as effective factor on keeping the total RNA and DNA contents steady, by preventing the nucleic acid synthesis. As a result, it has been found that, in the headed stage of cultivars the increase in the total RNA and DNA was generally important for the cold acclimation and hardiness. Jung, Shih and Shelton (1967b) indicated that the cold hardiness developed more rapidly, and was kept high for a long time, by spraying purine and pyrimidine solutions on alfalfa plants. Their findings prove that nucleic acids are important for cold acclimation and hardiness.

In literature, the relationship of free amino acid content with cold acclimation and hardiness does not seem to be clear, as it is explained below. Our findings showed that the increase of free amino acid content is related with acclimation and hardiness only in Bayraklı cultivar. The relationship obtained in this cultivar with free amino acid nitrogen content may be provided through protein synthesis which will be explained as follows. In some of the studies done in literature various woody and herbaceous plants were used as experimental material and their results support the existence of relationship between the increase of amino acid levels and hardiness (Wilding, Stahmann and Smith, 1960; Pauli and Zech, 1964; Gusta and Weiser, 1972). In some other studies, which had also supported results, there were also realtionships between acclimation, hardiness and amino acid decrease, as we obtained in the headed stage of Samsun top and Yeşilköy cultivars and in the seedling stage of all the three cultivars (Li, Weiser and Van Huystee, 1965, 1966). In literature, there is only one report on cabbage. Le Saint (1966) put forward that a decrease of amino acid nitrogen was related with development of hardiness. The patterns of relationship of the changes show differences in literature and in our studies with the cultivars and even in their samples. This shows that, examining amino acids totally is not a good parameter for cold acclimation and hardiness. As a matter of fact, in alfalfa Wilding, Stahmann and Smith (1960) who had examined the amino acids individually by chromatography and in red-osier dogwood Li, Weiser and Van Huystee (1965) who had examined the changes of 15 amino acids and amides and found that some of them decreased, some of them increased and some of them fluctuated very little in relation with hardening. Increasing and decreasing of a specific amino acid can affect the synthesis of various proteins in different ways. On account of this, the role of individual amino acids in acclimation and hardiness of cabbage, and their roles in protein synthesis should be examined.

Accordingly, the soluble protein increase is related to the hardiness of Bayraklı and to the acclimation and hardiness of Yeşilköy cultivars, while the soluble protein content decreased in Samsun top cultivar. Our results related with Bayraklı and Yeşilköy cultivars support the idea of some researchers, who used woody and herbaceous plants (Siminovitch and Briggs, 1949; Pauli and Zech, 1964; Li, Weiser and Van Huystee, 1965; Jung, Shih and Shelton, 1967a; Gerloff, Stahmann and Smith, 1967; Sakai and Yoshida, 1968; Gusta and Weiser, 1972). Their idea was that there was a relationship between hardiness and soluble protein content. Yet, in cabbage, Le Saint (1966) found that the decrease in protein nitrogen increased hardiness; Harper and Paulsen (1967) found that the soluble protein decreased while the hardening of "Pawnee" wheat crown tissue was being formed. Their findings support our results related to the decrease in Samsun top cultivar. Moreover, the literature has put forward contradictory results, such as no change of protein content in the leaves of grapefruit during the hardiness development and decreasing of the soluble protein content under the artificial hardiness conditions (Young,1969). We also observed the existence of different relationships among the cultivars used. Different patterns of soluble protein changes in the cultivars we examined, can be evaluated individually as follows.

Decreasing soluble proteins in Samsun top cultivar may be related to the increase of proteins bound to membrane; that shows the synthesis of proteins which have lower entropy. As a matter of fact, the literature has defined that decrease in soluble protein was the result of increasing of proteins bound to membranes and that they were more hydrophobic than soluble proteins (Chou and Levitt,1972). On the other hand, the metabolism can be directed to a specific purpose only in this way. Decomposition of proteins as a result of frost effect, in Yeşilköy cultivar, but it was found out that the changes of protein content which was related to acclimation and hardiness of Yeşilköy cultivar were very little. Although soluble protein content changed in relation to hardiness of Bayraklı cultivar its level did not reach the control level. It is significant if compared with the acclimation stage. These increases are related to the synthesis of new enzymes; new protein synthesis which are necessary for acclimation and hardiness. As a matter of fact, Cox and Levitt (1969) put forward that new proteins which were not denatured at hardening temparatures, were necessary for hardiness, were synthesized. It is logical to think that some of the proteins are not denatured in cold: During the cold acclimation sugars are formed by hydrolization of starch; by the activity of several enzymes, which are in working state. It has also been observed in the protein increase. Considering the denaturation of enzyme proteins results the lost of activities this conclusion can be drawn (Chou and Levitt, 1972).

But, a relationship can not be found with the soluble protein content in the seedling stages of the chilled samples of the cultivars. The acclimation occurring as a result of being chilled in the young seedlings, depends much on the carbohydrate changes. Due to not obtaining the general relationship between the soluble protein and cold acclimation and hardiness, we think that protein synthesis rate, proteolitic activity and soluble and bound protein content should be comparatively examined during the cold acclimation and hardiness.

Literature explains that the stopping of the growth was necessary for hardiness at low temperatures in woody plants (Weiser, 1970). It was explained that cabbage leaves that complete their growth had maximum hardiness at low temperatures where hardiness develop (Cox and Levitt, 1969). In woody plants, it has been found that there is generally a relationship between cold hardiness and the higher amounts of soluble protein, nucleic acid and amino acids. Our findings show that dormant woody plants in which the growth ceases and non-dormant cabbage plant needs almost the same metabolites to develop cold acclimation and hardiness to stay alive. We also found that there was a relationship between cold acclimation and the same biochemical parameters in the cabbage cultivars which were headed in the cold acclimation and hardiness period, and accordingly completed their growth. In the three cabbage cultivars, it has been found that the parameters such as total RNA and DNA, free amino acid nitrogen and soluble protein can also be related to cold acclimation and hardiness. But these examined parameters show different patterns in different cabbage cultivars. For this reason, cold acclimation and hardiness of cabbage cultivars should be individually examined, but not in cabbage plant. However, there can be some general relationships.

Our results show that the most hardy cultivar is Bayraklı cabbage and that at the second level there are Samsun top and Yeşilköy cultivars. Considering the results of all parameters measured, there is a relationship which can be generalized: A total increase in the metabolites, during the cold acclimation and hardiness stages.

## CONCLUSIONS

A– To select and treat the most appropriate cultivar:

1– In our research the increases of nucleic acids (DNA and RNA) have been related to cold acclimation and hardiness. As a matter of fact, in alfalfa (Jung, Shih and Shelton, 1967a; Shih, Jung and Shelton, 1967), in apple (Li and Weiser, 1969), in spring and winter wheat (Sarhan and D'aoust, 1975), in boxwood (Gusta and Weiser, 1972), in pseudoacacia (Siminovitch, 1963) it has been observed that, there was the same relationship. For this reason, it can be generalized that, increases in nucleic acids provide cold acclimation and hardiness in plants, including cabbage.

2– Any hardening mechanisms that are characteristic to cabbage plant, could not be found.

3– The parameters we examined showed different relationships in different cabbage cultivars. Parameters which showed individual patterns of changes in every cultivar during cold acclimation and hardiness, are the increases in soluble protein and free amino acid nitrogen.

4– Following suggestions can be offered for the cultivars examined, from the stand point of horticulture. The effect of amino acids should be examined by spraying amino acid solutions to Samsun top and Yeşilköy cultivars. Because the amount of amino acids is very little in these cultivars. The same procedure can be relevant to Bayraklı cultivar if low concentrations are used; because high contents of amino acids are found in Bayraklı. The nitrogen fertilization can also be tried in autumn, to obtain the increases of amino acid and soluble protein levels.

B– Following suggestions can be offered regarding genetic improvement studies:

When choosing the cabbage and most likely the other vegetables species, to be grown in winter and the new variety production, it seems necessary to choose the cultivars which can especially store high nucleic acids, amino acids and soluble protein. Improvement studies should be done to obtain cultivars having these properties.

C– The following suggestions can be offered from the stand point of cabbage growing.

1– In our experiments, two seed sowing and seedling planting periods used: June and October. The changes related to biochemical parameters 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 these plants can be left in the field where the lowest temperature is at least approximately – 15 °C.

2– Bayraklı cultivar which is found to have a higher cold acclimation and hardiness level seems to be suitable for this application.

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