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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.

## I-Soluble carbohydrate and starch fluctuations


by

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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.

## I-Soluble carbohydrate and starch fluctuations

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**ABSTRACT**

The relation of cold acclimation and hardiness with starch and soluble carbohydrates in various plants has long been known. In this study, this relation has been investigated on non-dormant cabbage. The effect of cold on three agricultural forms of white head cabbage, namely Samsun top, Yeşilköy and Bayraklı has been investigated in different development stages. For this purpose the plants were sampled during seedling and headed phases. The plants to be sampled in seedling stage were planted in October and those to be sampled at headed stage were planted in June. It was observed that three cultivars of cabbage planted in June froze but that planted in October did not freeze, namely chilled.

A considerable increase in glucose, fructose and some increase in saccharose levels were related to cold hardening of headed Samsun top, while similar increases were in correlation with cold acclimation and hardiness of headed forms of Yeşilköy and Bayraklı.

The increases in glucose, fructose and saccharose levels of the chilled samples of Samsun top, Bayraklı and Yeşilköy seedlings have been found related to hardening. The changes in fructosans have been found related to only cold acclimation of headed Samsun top while they have not been related to seedlings and headed forms of Bayraklı and Yeşilköy. In the headed and seedling stages total starch content decreased and this development has been related to cold acclimation and hardiness.

The relationship between a decrease in starch and a corresponding increase in soluble carbohydrates and cold hardiness suggests that these substances are important factors in the mechanism of development of cold hardiness in all of these cultivars.

## INTRODUCTION

The opposite relation between growth and frost hardiness has long been observed in dormant woody plants. However, in non-dormant herbaceous plants, development of hardening has long been paralleled to growth rate under cold stress (COX and LEVITT, 1969).

The aim of this study is to examine the physiological and biochemical mechanisms of cold acclimation and frost hardiness in herbaceous plants which do not have dormancy period.

In the studies related to cold acclimation and hardiness, there are various parameters related to this subject being used of herbaceous plants such as grasses, English ivy, turfgrass, potato, alfalfa, winter wheat and of woody plants such as mulberry tree, black locust tree, poplar, box elder, red-osier dogwood, apple, oak, birch, azalea, grapefruit. These results can be put together as discussed below.

It has been found out that the changes in nitrogen metabolism in the development and protection of cold hardiness were important (COLEMAN, BULA and DAVIS, 1966). However, the relationship between hardiness and the amount of total nitrogen seemed to be contradictory, while DROZDOV and SYCHEVA (1965) found that higher nitrogen fertilization increased hardiness in the herbaceous plants, HOWELL and JUNG (1965) explained that phenomena as the plants fertilizated with little nitrogen were more hardy to cold than the ones fertilizated with much nitrogen. On the contrary, JOINER and ELLIS (1964) also put forward that the supply of nitrogen did not change cold hardiness in woody plants. 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 found out that in the cold hardiness the contents of RNA, DNA and soluble protein in hardy herbaceous plant species might be greater that those in tender herbaceous plant species (JUNG, SHIH and SHELTON, 1967). The relationship of nucleic acids with cold hardiness has studied because of their importance in protein synthesis.

As protein were produced because of the polimerization of amino acids, the relationship of amino acids with cold hardiness was studied.

Although LI, WEISER and VAN HUYSTEE (1966) have found out 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 herbaceous plants during the cold hardening period were much higher in hardy species (PAULI and ZECH, 1964). Thus, it has been shown that protein metabolism and related to this the changes in RNA, DNA and amino acids were responsible for the development of hardiness in woody and herbaceous plants.

The first phase of acclimation starts in fall and develops in the low positive temperature ranges. Under these conditions, large amounts of sugars accumulate while the rate of photosynthesis is constant and growth rate is decreasing (TUMANOV, 1931; ANDERSON, 1944). MULLER-THURGAU (1882) firstly demonstrated that there was a positive correlation between sugar content and frost hardiness. SAKAI (1962) has shown that sugar accumulation could protect cabbage tissues from injury by preventing intracellular freezing. Many investigators have shown a pronounced parallelism between sugar content and the consequent high osmotic potentials of the cell sap and frost hardiness and correlated cold resistance directly with high dry matter content of cabbage (KOHN and LEVITT, 1965; SAKAI and YOSHIDA, 1968). Although the correlation between sugar content and frost hardening has also long been stated (YOUNG, 1969). STEPONKUS and LANPHEAR (1968) drew attention to the lack of parallellism between sugar content and hardiness and interpreted this unparallellism as indications of acclimation was not merely the result of an accumulation of sugars or an osmotic effect per se and under normal conditions, the level of carbohydrates was not limiting the rate of development or degree of cold acclimation.

A decrease in starch and a corresponding increase in sugars has long been associated with cold acclimation and hardiness (LI, WEISER and VAN HUYSTEE, 1965, 1966) and various sugar forms have been known to accumulate in plants (TRUNOVA, 1965, OGOLEVETS, 1964, 1966). The researches have explained that a decrease in starch and a parallel increase in sugars were important to cold hardening and freezing resistance as a protection of the proteins by sugars that accumulate on hardening.

Although KAWANA et al., (1964) have explained that nutrient salts having an important role in plant growth were also very important in the development of the cold hardening; SAKAI (1960) has stated that various organic substances are protective aganist freezing and that as for inorganic salts they do not have any protective roles. While LYNCH and GOLDWEBER (1956) stating that there is not any relationship between phosphorus levels and frost hardiness in woody plants; LI, WEISER and VAN HUYSTEE (1966) found that total inorganic phosphorus decreased and that total and organic phosphorus increased.

It has also been stated that potassium was able to increase the cold hardening by accelerating amino acid and carbohydrate synthesis (HAYDEN et al., 1969), but COOK and DUFF (1976) have explained that potassium fertilization has not increased the accumulation of carbohydrate and the freezing tolerance in herbaceous plants. Although JOINER and ELLIS (1964) found that the 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 (MARSHALL, 1969). TRESHOW (1970) also stated that the amounts of much sodium and calcium had increased low temperature injury. As seen, there is not agreement on cold hardening effects of nutrient salts in woody and herbaceous plants.

Although the relationships of protein, nucleic acid, amino acid, sugar and some nutrient salts with cold acclimation and hardening have been examined by using different woody and herbaceous plants, yet, there are not any studies in which these parameters were examined in a single experimental design. Additionally, the effects of nutrient salts on hardening have never been examined in cabbage, particularly.

Considering that, cold acclimation and hardiness may have been resulted from the interaction among these parameters, the aim of our study is to examine biochemical mechanism containing soluble protein, total RNA and DNA, free amino acid nitrogen, various soluble carbohydrates and starch in cabbage, which is characterized by having a nondormant stage differing from woody plants.

The main aim of our study is also to examine the relationships between the important nutrient salts of plant metabolism such as sodium, potassium, calcium, phosphorus and total nitrogen and the physiological mechanism of cold acclimation and hardiness in cabbage. There are no studies belonging to nutrient salts which are relevant to cold acclimation and hardiness of cabbage and the results related to nutrient salts obtained from woody and herbaceous are contradictory. Moreover, it has been noticed that most of the studies regarding nutrient salts have been done, considering the principles of fertilization.

In order to get the useful information for application in agriculture, it was aimed to determine a hardy cultivar using the cabbage seeds which have been registered by Agricultural Research Institutes. By choosing cultivars in this way, we will try to evaluate physiological parameters to be used in seed improvement. To obtain the results to be evaluated clearly in a comparative system, the same methods on three cabbage cultivars were examined.

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

## MATERIAL AND METHODS

### PREPARATION OF MATERIAL

Three white head cultivars of cabbages were used as experimental materials.

To examine the effect of cold on the different physiological development stages of cabbage, the plants were sampled during seedling and headed phases. The plants to be sampled in seedling stage were planted in October and those to be sampled at headed stage were planted in June. The plants were left under the effect of cold in the garden of Science Faculty of Ankara University. The experiments were made in 1976.

The seeds were germinated in pots (r: 25 cm, h: 5 cm) in a glasshouse. The soil was a mixture of vegetable mould, barnyard manure and garden soil. After a week, the seedlings were transferred outside for acclimation and after two weeks, they were transferred to a plot in the field (L: 6m, W: 4m).

The soil was manured with barnyard manure 3–4 tons per acre. The seedlings were planted in 80 cm rows with a distance of 60 cm. The preparation of soil and growing of plants were made according to GÜNAY (1975).

## THE METHOD OF SAMPLING

The cabbages planted in June and October were sampled in December. When the samples were taken, the cabbages planted in June were headed and those planted in October were seedlings with 5–6 leaves. These sample groups were termed "control". It was observed that the controls were unaffected from cold stress and they could survive in minimum temperature of —9.6°C in December.

The plants again were sampled in the middle of January. The young head samples of three cultivars which were planted in June, termed "frozen". These frozen young head cabbages were sampled and classified according to the degree of injury observed on leaves as "unaffected" and "affected". A part of headed cabbages were left until the end of January in the garden and then were sampled. This sample group was termed as "affected aged head". Affected and unaffected heads were differentiated by examining them by hand and by looking at their appearence during the sampling. Also the number of the injured leaves surrounding the frozen heads were counted. The variation of the numerical values of our observations related with sampling of the headed cultivars planted in June are summarized in Table 1.

It was observed that three cultivars of cabbage seedlings planted in October did not freeze but chilled. This sample group was termed "chilled".

## PRESERVATION OF SAMPLES AND PREPARATION TO CHEMICAL ANALYSIS

A part of samples were immediately transferred and stored in deep-freeze for chemical analysis. Then these samples were freeze-dried and were ground in the mill and stored in a desiccator on calcium chloride.

Sixty plants of each cultivar were grown and thirty of them were taken as "controls" and "aged head" samples. All of the rest were taken as "frozen unaffected and affected young head" samples. In order to lyophilize the tissues in short periods the thin slices of each sample were freeze-dried and ground then they were homogenized and the representing samples of each group were obtained.

Table 1– The variations of the numerical values of observations related with samplings of the headed cultivars planted in June.

| Cabbage cultivars | Sample name | The number of used cabbage | Affected |
|---|---|---|---|
| White head cabbage (Samsun top) | Control | 15 | —— |
| | Frozen unaffected young head | 14<br>30 | 5 affected outmost leaves |
| | Frozen affected young head | 16 | 8 affected outmost leaves |
| | Frozen affected aged head | 15 | 7 frozen inmost leaves |
| Native white head cabbage (Yeşilköy) | Control | 15 | —— |
| | Frozen unaffected young head | 13<br>30 | 6 affected outmost leaves |
| | Frozen affected young head | 17 | 9 affected outmost leaves |
| | Frozen affected aged head | 15 | 6 frozen inmost leaves |
| Bayraklı cabbage | Control | 15 | —— |
| | Frozen unaffected young head | 15<br>30 | 5 affected outmost leaves |
| | Frozen affected young head | 15 | 7 affected outmost leaves |
| | Frozen affected aged head | 15 | 8 frozen inmost leaves |

The vegetative part of the seedlings were taken and analyzed. A hundred of seedlings were taken at least as controls or chilled samples. The samples were homogenized and representing samples were taken from each group.

## METHODS

### SOLUBLE CARBOHYDRATE DETERMINATION

The extraction of soluble carbohydrates was made according to the method of SMITH (1967, 1968). The extractions of glucose+saccharose and fructose+saccharose were made with 80% ethanol and ethanol was evaporized and the residue was solved in water. The extraction of long chained fructosans was made with water. The aliquots of glucose+ saccharose, fructose+saccharose and fructosans were determined by colorimetric method using anthrone reagent according to HALHOUL and KLEINBERG (1972).

## STARCH DETERMINATION

The extraction of starch was made according to PUCHER et al. 1955. The determination of starch was made according to the method developed by McCREADY et al. 1950.

All of the experiments were repeated at least four times.

Significant at 5% level.

## RESULTS

## SAMPLING

The sampling method used in this study has been developed as described below. This method was used in order to examine the severity of cold injury on the different physiological development stages. The cabbages planted in June were in headed stage and those planted in October were seedlings with 5–6 leaves at the begining of the cold period. The samples of headed cabbages were taken in January and were grouped as young-affected, unaffected and aged. The control samples of each cultivar were taken in December, while the minimum temperature was —9.6°C, but there was no visible injury on the plants. Sampling of the controls has been made according to GÜNAY (1975) who observed that the cabbages were tolerant to low temperatures down to —10°C. The investigator explained that the cabbages were hardened by —10°C in one day or sometimes in three days.

It was observed that all of the controls of headed cabbages protected their colour and did not lose their freshness. The seedlings which were green did not show any signs of drying and withering. It was seen that the cabbages were not affected by low temperatures which were down to —10°C. But the headed cabbages affected by cold which were planted in June and the seedlings planted in October when sampled in January. The lowest temperature of this month was —15.6°C. when the samples were taken.

a) The sampling of cultivars planted in June and exposed to cold:

The headed cabbages were sampled after being exposed to cold and they were labelled as "young head" and "aged head" samples. The samples as defined below were exposed to cold for a short period and were labelled as "young head" and the others were exposed to cold for longer periods were labelled as "aged head". The headed cabbages were frozen because of the existence of in their structure. But they were alive because they did not show any sings of death tissues such as the changes of colour and decaying. These frozen headed samples were labelled as "frozen young head". The frozen young head cabbages were resampled considering the degree of the signs of cold injury. Five or six upper layers of leaves of the heads were affected by cold and frost as their colour turned to light brown. They became soft and rotted. The inner part of heads were frozen but alive. This sample group was labelled as "unaffected". The inner layers did not show this kind of symptoms although they were frozen. Therefore, it was established that they were alive.

The other sample group where the decaying occurred in the seven to nine layers of upper leaves were affected by cold and the inner part was frozen but alive was labelled as "affected". In this sampling group, it was observed that almost all of the seven or nine leaf layers were to brownish, soft and decayed. But in the inner part, not signs of injury was observed although they were frozen. They were alive. Thus, the cultivars of cabbages planted in June affected by cold and have been labelled as "frozen unaffected young head" and "frozen affected young head". A group of headed cabbages planted in June have been left in the garden until the end of January and as a result of this, they have been affected by cold for about fifteen days more than the younger samples. These headed cabbages were labelled as "aged head". They were called as "aged head" because they became senescence under the effect of cold. The physiological age was the matter in question because these samples were also planted in June. Being a little longer of the duration of their affected periods by cold has made these samples aged when they are compared to the young head samples. It is known that all kinds of stresses, chilling and freezing have the effects that make the plants aged, senesced and kill them (FUCHS, 1970). As a result of aging, those which have gained the ability to endure the cold will survive, those which have not gained this ability will die. Not gaining the ability of the hardiness to the cold is also the cause of senescence. Senescence also destroys membranes and consequently the loss of permeability occurs, this causes the loss of various metabolites. In the present study it has been seen that the aged head samples were affected by cold at most. It was established that only six or eight leaves in the inner part of head were not frozen and in general all of the surrounding leaves were affected, jellous and decayed. For this reason, this sample group was labelled as "frozen, affected aged head". In this sample group, it has been seen that nearly all plants belonging to the three varieties were equally affected by cold.

b) The sampling of cultivars planted in October and exposed to cold:

It was seen that three varieties of cabbage seedlings planted in October and harvested in January were not frozen but chilled. For this reason the seedlings planted in October were labelled as "chilled". Because the snow layer protected them from the severe effect of freezing temperatures down to —15.6°C, thus prevented the structural water to freeze. The injury effect of low temperatures over freezing point was defined as "chilling" (MOLISCH, 1897). As a result of chilling in the seedlings it was seen that the most aged three or four leaves were half-dried but the two upper young leaves were fainted. That's why this effect was reversible. When the normal conditions were provided the plant acquired its liveliness again. It was seen that during the control sampling the seedlings with five or six leaves did not grow in the chilly stage too and remained with five or six leaves. The results of cultivars of white head (Samsun top), native white head (Yeşilköy) and Bayraklı are summarized in Table 2. Moreover the symbols used in figures have been explained in Table 2.

Our observations during the sampling showed that very evident and important differences did not exist during the cold acclimation and and hardiness among the cabbage cultivars used. These samples were in the stages of acclimation and hardiness. The chilled samples of cultivars planted in October were the samples of acclimation because the chilling temperatures fell down to minus degrees down to —15.6°C later. This pattern of changes in tempeature provided the plants to harden. The unaffected young head samples of the cultivars planted in June were also acclimated because the headed ones were not affected by —15.6°C cold treatment.

Table 2– The samplings of White head cabbage (Samsun top), Native white head cabbage (Yeşilköy) and Bayraklı cabbage cultivars.

| Cultivars | Planting | Sampling | Temperature during the sampling(°C) | Samples name | Physiological development stage of samples | Affection +...Affected —...Unaffected | Affection degree | Sample name |
|---|---|---|---|---|---|---|---|---|
| White head cabbage (Samsun top) | June | December | – 9.6 | Control | Headed form | — | —— | C |
| | June | January | –15.6 | Frozen | Young head | — | 5 outmost leaves | (YH,—), F |
| | June | January | –15.6 | Frozen | Young head | + | 8 outmost leaves | (YH,+), F |
| | June | January | –15.6 | Frozen | Aged head | + | 7 frozen inmost leaves | (AH,+), F |
| | October | December | – 9.6 | Control | Seedling with 5–6 leaves | — | —— | C |
| | October | January | –15.6 | Chilled | Seedling with 5–6 leaves | + | Half-drying in the 3–4 aged leaves | Ch |
| Native white head cabbage (Yeşilköy) | June | December | – 9.6 | Control | Headed form | — | —— | C |
| | June | January | –15.6 | Frozen | Young head | — | 6 outmost leaves | (YH,—), F |
| | June | January | –15.6 | Frozen | Young head | + | 9 outmost leaves | (YH,+), F |
| | June | January | –15.6 | Frozen | Aged head | + | 6 frozen inmost leaves | (AH,+), F |
| | October | December | – 9.6 | Control | Seedling with 5–6 leaves | — | —— | C |
| | October | January | –15.6 | Chilled | Seedling with 5–6 leaves | + | Half-drying in the 3–4 aged leaves | Ch |
| Bayraklı cabbage | June | December | – 9.6 | Control | Headed form | — | —— | C |
| | June | January | –15.6 | Frozen | Young head | — | 5 outmost leaves | (YH,—), F |
| | June | January | –15.6 | Frozen | Young head | + | 7 outmost leaves | (YH,+), F |
| | June | January | –15.6 | Frozen | Aged head | + | 8 frozen inmost leaves | (AH,+), F |
| | October | December | – 9.6 | Control | Seedling with 5–6 leaves | — | —— | C |
| | October | January | –15.6 | Chilled | Seedling with 5–6 leaves | + | Half-drying in the 3–4 aged leaves | Ch |

C : Control
YH: Young head
AH: Aged head

F : Frozen
Ch: Chilled

(—): Unaffected
(+): Affected

Consequently the chilled samples of seedlings planted in October and unaffected young head samples planted in June are the samples which acclimated. The affected young head and affected aged head samples which were planted in June are also hardened samples.

## THE CHANGES IN THE CONTENT OF SOLUBLE CARBOHYDRATE AND STARCH

### 1- THE CHANGES IN THE CONTENT OF SOLUBLE CARBOHYDRATE

In literature it has been explained that various soluble carbohydrates increased during cold acclimation and hardiness (OGOLEVETS, 1966; YOUNG, 1969). The results we obtained during cold acclimation and hardiness had a relationship with various soluble carbohydrate levels and showed a relationship with cold acclimation and hardiness. Glucose+saccharose and fructose+saccharose fractions in the frozen unaffected young head of Yeşilköy and Bayraklı cultivars cold acclimation increased glucose, fructose and saccharose (Figure 1 and 3). Especially the increase in glucose was important as for the hardiness period of headed Yeşilköy cultivar. There was no relationship between the amount of glucose+saccharose in samples of the frozen, affected young head and aged head samples or frozen affected young head of Bayraklı cultivar. Although the frozen affected aged head has shown an increase when compared to the control glucose+saccharose content is less than found in the acclimation period. In samples of hardening of three cultivars, it was found that glucose was the main effective agent, decreased in acclimation while fructose increased (Figure 1 and 3) during the development of hardiness. During hardening of Yeşilköy and Bayraklı cultivars in the frozen affected young head samples no relationship between fructose+saccharose content could be found. The reason of the increase in the frozen affected aged head samples can be explained in terms of hardening: it did not develop well enough to increase glucose+saccharose and fructose+saccharose content. Samsun top cultivar shows a different behavior from that of Yeşilköy and Bayraklı cultivars. It was found that the frozen unaffected young head sample which is corresponding to acclimation period of headed Samsun top cultivar while glucose+saccharose and fructose+saccharose content is not related in hardening of frozen affected young head and frozen affected aged head samples (Figure 1 and 3).

In the headed plants decreasing of glucose+saccharose and increasing of fructose+saccharose content means decreasing of glucose/fructose ratio and can be taken as indication of senescence developing as a result of aging promoted by cold. During the period of senescence, it is known that glucose decreases but fructose increases in senescence as a matter of fact (MILLER, 1958). He showed that glucose decreased but fructose increased in various aged plant organs. Considering that this change might accompany aging in frozen affected aged head samples of the headed cultivars. By examining how the rate of glucose+saccharose/fructose+saccharose changed the following results were obtained by comparing this ratio to the control. This ratio in Samsun top is 6.3/4.3 = 1.4 and in Bayraklı cultivar it is 5.7/3.6 = 1.5. Thus it can be said that these are the least affected cultivars. As for Yeşilköy the ratio is 5.4/2.6 = 2.07 and shows that this cultivar is the mostly injured, the most tender cultivar.

A relationship with glucose+saccharose content was found in the chilled samples which are in the acclimation periods (Figure 2). During this period the Samsun top and Bayraklı cultivars showed no relationship with the fructose+saccharose content but glucose+saccharose showed relation in these two cultivars (Figure 4). In the seedling form of Yeşilköy cultivar relationship with fructose+saccharose content has also been obtained in the acclimation period, the increases of glucose, fructose and small amount of saccharose are related with in this cultivar. In the chilled acclimation samples of the seedlings of cultivars when the ratio of glucose+saccharose/fructose+saccharose were examined and compared to the controls, it is seen that the acclimation in Samsun top and Bayraklı cultivars was higher. Although there was a similar relationship in Yeşilköy the ratio of glucose+saccharose/fructose+saccharose showed that this relationship was not sufficient for a good development of acclimation. The ratios are as a matter of fact 1.9/7 = 0.27 for Samsun top, 1.6/4 = 0.4 for Yeşilköy and 2/45 = 0.04 for Bayraklı. Glucose is dominant sugar in all of these cultivars and seems to be the sugar which is effective in acclimation.

It has been found that there was no relationship between the content of fructosans and cold acclimation or hardiness of the headed

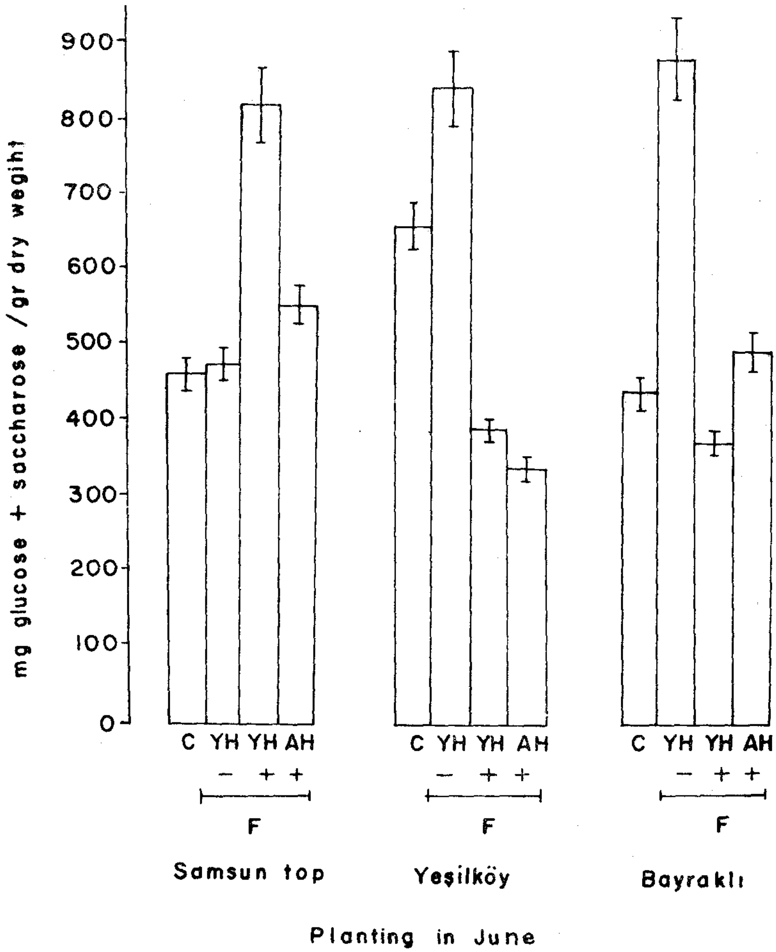


Figure: 1– The content of gluctose + saccharose in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

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

frozen samples of the three cultivars. It can be stated, on the other hand, that the content of fructosans is related to development of acclimation only in frozen unaffected young head samples of Samsun top cultivar (Figure 5). Supporting this hypothesis, in the acclimation stage of Sam-

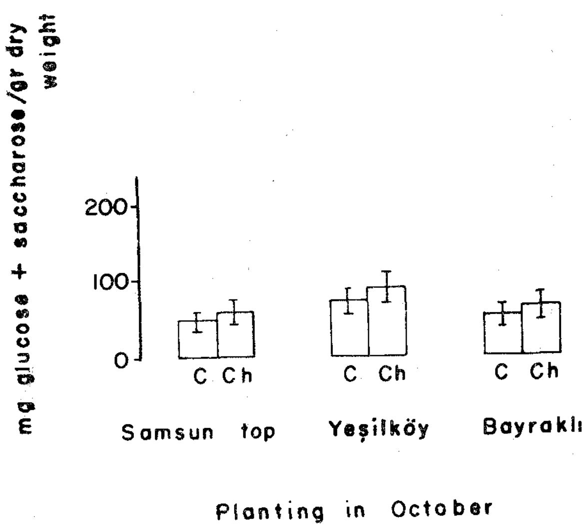


Figure: 2– The content of glucose + saccharose in control and chilled samples of the seedlings of Samsun top. Yeşilköy and Bayraklı cabbage cultivars.
C: Control Ch: Chilled

sun top, no relationship with fructose+saccharose and glucose+saccharose parameters was obtained. In Yeşilköy and Bayraklı cultivars a relationship has not been found because fructosans turned fructose.

In the chilled samples of the seedlings of cultivars it can not be said that the fructosan content was related to the cold acclimation and hardiness (Figure 6). This also shows that the other sugar forms were effective in the above mentioned samples.

## 2– THE CHANGES IN THE CONTENT OF STARCH

As explained in literature, there is a decrease in starch and a corresponding increase in soluble carbohydrate contents during the period of cold acclimation and hardiness (LI, WEISER and VAN HUYSTEE, 1966). The results obtained in the present study during the cold acclimation and hardiness have a relationship with a decrease in starch content when compared with the controls and this decrease in starch can be related to cold acclimation and hardiness. Best relationship was obtained in headed samples of Bayraklı cultivar which is known as a hardy cultivar against cold (Figure 7). It can be concluded that the starch

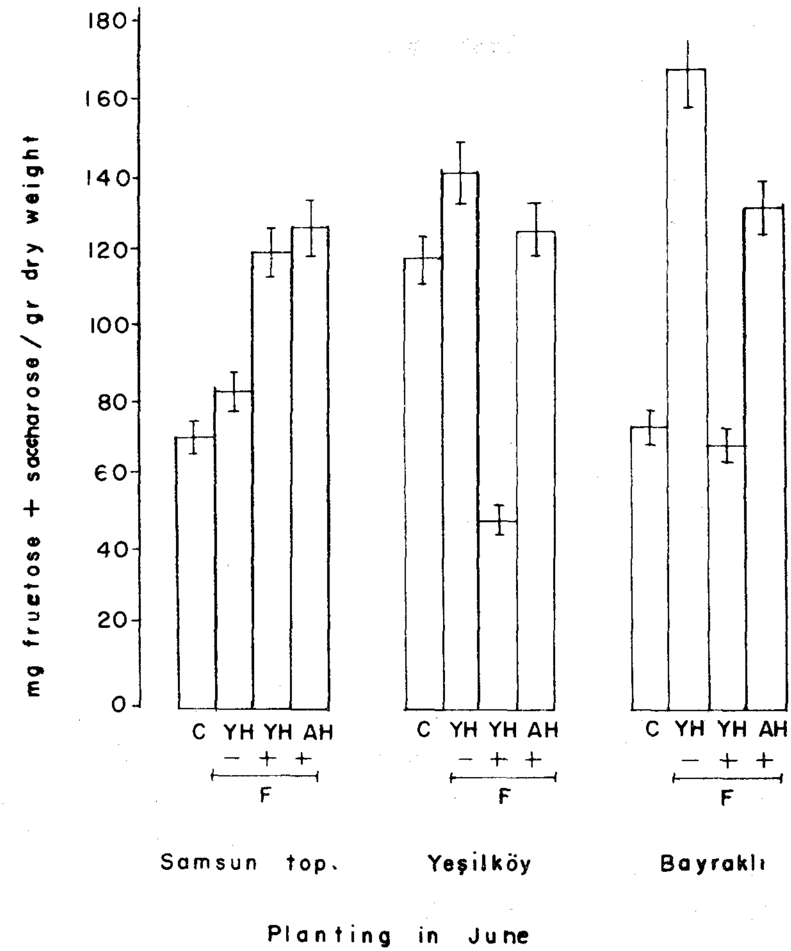


Figure: 3– The content of fructose + saccharose in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.
of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

content was related with hardening in the frozen unaffected and affected young head samples of Samsun top and Yeşilköy cultivars but not related in affected aged head samples of them, that is, it increased in these samples (Figure 7).

While there is a relationship with the starch content in the seedling samples of Samsun top and Bayraklı cabbages, this relationship does

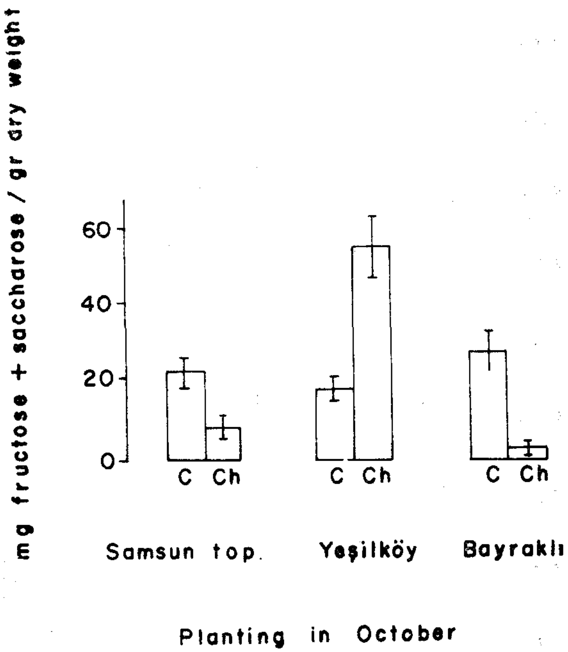


Figure: 4– The content of fuctose + saccharose in control and chilled samples of the seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultiyars

not exist in Yeşilköy cultivar. Starch increased in the seedling form of Yeşilköy (Figure 8).

If it is summarized, the role of sugars in the hardiness of cabbages against cold stress, the effects of chilling and frost must be considered separately. Because as it has been mentioned above senescence development by the effect of cold increases the sugars but hardiness development prevents this. Because of this the roles of various soluble carbohydrates in hardiness and the changes which are related to senescence should be commented separately. The relationships with the hardiness development of cabbage plant should be examined according to this main factor. While the considerable increase of glucose, fructose and little increase in saccharose is accompanying cold acclimation and hardiness of Yeşilköy and Bayraklı cabbage cultivars, this increase in Samsun top cultivar was related only with hardiness. The high ratio of glucose+sacc-

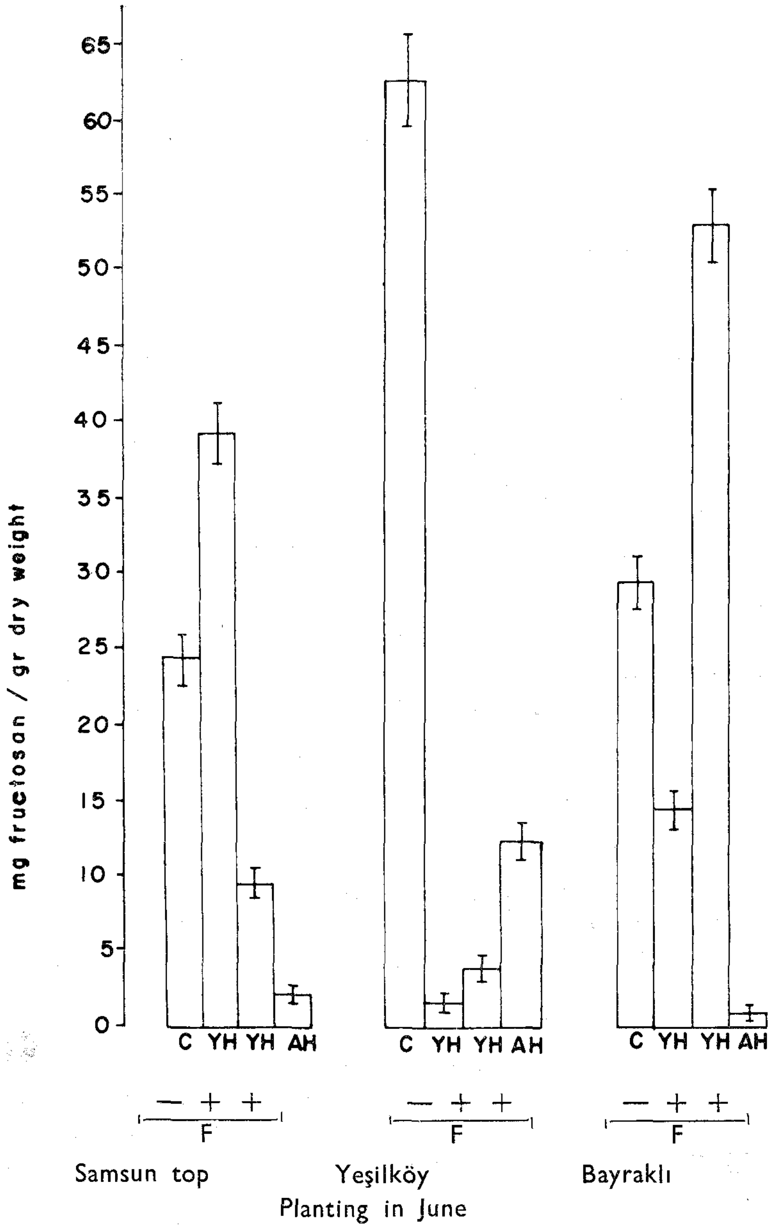


Figure: 5– The content of fructosan in control and frozen samples of the headed plants of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

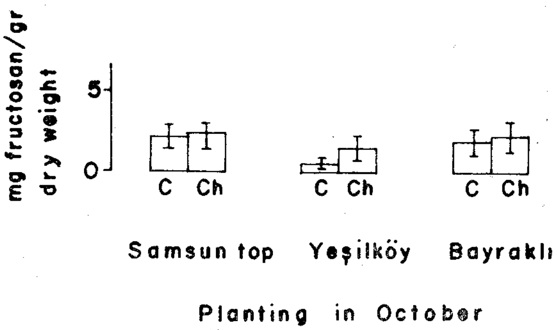


Figure: 6– The content of fructosan in control and chilled samples of the seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

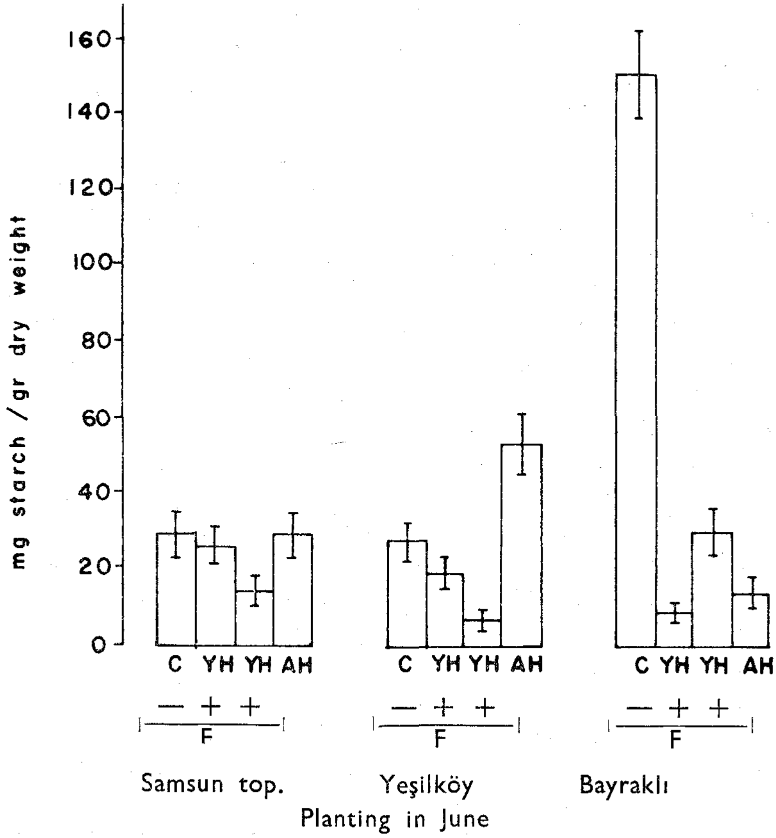


Figure: 7– The content of starch in control and frozen samples of the headed plants of Samsun top. Yeşilköy and Bayraklı cabbage cultivars.

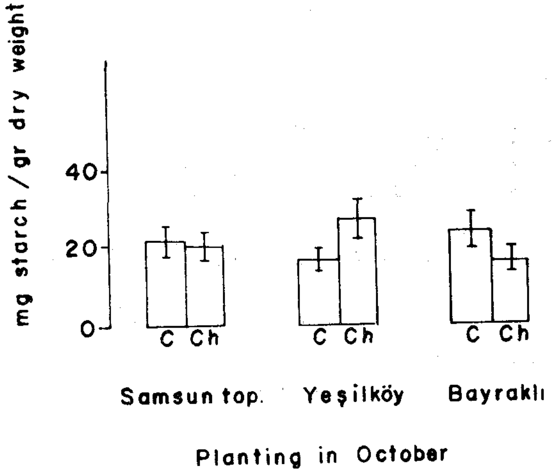


Figure: 8– The content of starch in control and chilled samples of the seedlings of Samsun top, Yeşilköy and Bayraklı cabbage cultivars.

harose /fructose+saccharose during the period of senescence means that Bayraklı and Samsun top cultivars are the least affected but that Yeşilköy cultivar is the most sensitive one. While it can be said that glucose+saccharose in the chilled samples of the seedling forms of Samsun top and Bayraklı cultivar and glucose, fructose and saccharose in Yeşilköy cultivar are related, the ratio of glucose+saccharose /fructose+saccharose shows that Samsun top and Bayraklı cultivars can acclimate very well and that the ability of acclimation of Yeşilköy cultivar is very little. While fructosan is not related to these developments in seedling and headed forms of cultivars it can be related only in the acclimation period of headed Samsun top cultivar. Although the total starch content is little whether in the headed or seedling forms of the cultivars, it has been found out that they were related to cold acclimation and hardiness. In this case the importance of polysaccharides is not very much but the increase of soluble carbohydrates is important although the relationships have been obtained both with glucose+saccharose content and fructose+saccharose content in these cultivars. It can be said that the main effective agent, is glucose. As a matter of fact on this subject SAKAI and YOSHIDA (1968) explained that the hardiness development against frost was the result of the increase in osmotic concentration in cabbage cells as a result of the increase in the sugar content.

## DISCUSSION

The results obtained in this research revealed the relationships of some biochemical parameters such as starch, fructosan, glucose+saccharose and fructose+saccharose with the cold acclimation and hardiness in the three cabbage cultivars used as plant material. When these parameters used in the evaluation of cold acclimation and hardiness of cabbage cultivars in the present study are compared to the results in literature the following evaluations can be done. In parallel to the decrease in starch content it has been found that the increases in glucose, fructose, saccharose contents and especially glucose have an important relation in acclimation and hardiness development in all three cultivars.

Actually in literature it has also been found that there is a decrease in starch content in parallel to the increase in soluble sugars (SAKAI, 1962; TUMANOV and TRUNOVA, 1963; OGOLEVETS, 1964, 1966; LI, WEISER and VAN HUYSTEE, 1965, 1966) and that the increase in the glucose, fructose and saccharose contents are important for cold acclimation and hardiness (TRUNOVA, 1965; YOUNG, 1969). But the relationship with the fructosan was found only in the acclimation stage of the headed form of Samsun top cultivar presently. As TRUNOVA (1965) explained that the oligosaccharides are the types of polyfructosans accumulated during the hardening of winter wheat. In our results the reason of the lack of relationship with fructosan and hardening can be explained by stating that the sugars related, accumulated in other forms. It can be said that best relationship was obtained in this study with soluble carbohydrates among examined biochemical parameters in the seedlings of cultivars. In this case the high level of soluble carbohydrates in seedlings can be taken as the indication of relatively high photosynthesis rate that was kept high even at low hardening temperatures. As a matter of fact SHATILOV, RACHINSKII and POLIKARPOV (1957) put forward that photosynthesis occurred in winter wheat at the temperatures below freezing point (below – 14 °C). If it is evaluated orderly it can be said that carbohydrates are consumed less in respiration in parallel to the increase in photosynthesis, that is, the respiration rate is low and as a result of this sugars accumulated rapidly and thus the hardiness developed.

WEISER (1970) stated that the stopping of growth was necessary for hardiness in woody plants at low temperatures. At low temperatures where hardiness develops it was shown, on the other hand, cabbage leaves which had completed most of their growth had maximum hardiness (COX and LEVITT, 1969). In woody plants it has generally been found that a large amount of soluble carbohydrates are related to the cold acclimation. Our results show that dormant woody plants where there is no growth and cabbage plant which is non-dormant need nearly the same metabolic substances so that they can develop the cold acclimation and hardiness and can survive. We also found a relationship generally between soluble carbohydrates and cold hardiness in the cabbage cultivars which are headed in the cold acclimation and hardiness period and thus which have completed their growth.

In summary, the increases in glucose, fructose and saccharose – especially glucose – contents have been found to be related to cold acclimation and hardiness. As a matter of fact in the plants of mulberry tree, birch, poplar, rose, oak, apple and black locust tree (SAKAI, 1960, 1962; SAKAI and YOSHIDA, 1968; OGOLEVETS, 1964, 1966), in winter wheat (TUMANOV and TRUNOVA, 1963; TRUNOVA, 1965; BABENKO and GEVORKYAN, 1967), in grapefruit (YOUNG, 1969) same relationship was found. At least for cabbage probably many other vegetable species which are most probably grown in winter, to choose the new cultivar for production, it is necessary to choose the cultivars which can store high levels of soluble carbohydrates.

As our experiments showed that if the results held on in June and October plantings have been mainly considered the changes in soluble carbohydrates obtained by the use of cultivars which are headed and planted in June, plantings can be performed in June and even in July. These plants can be left in the fields where the low temperature is about at least – 15 °C.

In general, Bayraklı cabbage cultivar in which we have found the highest cold acclimation and hardiness development is suitable for this application.

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## ÖZET

Çeşitli bitkilerde soğuğa uyum ve dayanıklılık olayı ile ilişkisi bulunmuş olan nişasta ve çözünür karbonhidratların kış uyku devresi olmayan lahanada soğuğa uyum ve dayanıklılık ile ilişkisi araştırılmıştır. Beyaz baş lahananın Samsun top, Yeşilköy ve Bayraklı çeşitlerinde farklı fizyolojik gelişme devreleri olan fide ve baş örneklerinde soğuğun etkisi incelenmiştir. Bu amaçla bitkiler fide ve baş bağlamış devrede örneklenmişlerdir. Fide devresinde örneklenmiş bitkiler Ekim ayında, baş devresinde örneklenmiş bitkiler de Haziran ayında dikilmişlerdir. Haziran ayında dikimi yapılmış üç lahana çeşidinin soğuk devrede donduğu, Ekim ayında dikimi yayapılmış olanların donmadığı fakat üşüdüğü gözlenmiştir.

Glukoz, fruktoz ve az olan sakkaroz artışı, baş halindeki Yeşilköy ve Bayraklı lahana çeşitlerinin soğuğa uyum ve dayanıklılık devresinde ilişkili olurken, baş halindeki Samsun top çeşidinde ise dayanıklılık devresinde ilişkili olmaktadır. Fide halindeki Samsun top, Bayraklı ve Yeşilköy çeşitlerinin üşümüş örneklerinde glukoz, fruktoz ve sakkaroz miktarındaki artışlar dayanıklılık ile ilişkili bulunmuştur. Fruktozan fide ve baş halindeki Bayraklı ve Yeşilköy çeşitleri ile ilişkili olamazken, sadece baş halindeki Samsun top çeşidinin uyum devresinde ilişkili olabilmektedir.

Çeşitlerin gerek baş gerekse fide devresinde azalan toplam nişasta miktarı soğuğa uyum ve dayanıklılık ile ilişkilidir. Çözünür karbonhidratlardaki artışa uygun olarak nişasta miktarının azalışı ile soğuğa dayanıklılık arasındaki ilişki, bu maddelerin üç lahana çeşidinde soğuğa dayanıklılığın gelişim mekanizmasında önemli maddeler olduğunu göstermektedir.