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A Review of Studies on Stress Physiology of Some Fruits and Vegetables

Atilla ERİŞ*
Nuray SİVRİTEPE**
H.Özkan SİVRİTEPE***

ABSTRACT

Turkey has different ecological regions and a wide range of species and cultivar richness in horticultural crops adapted to these regions. However, early spring frosts cause a considerable amount of yield loss in the regions that have temperate and subtropical climates. On the other hand, drought stress occurs in horticultural crops during summer, in the regions where precipitation is either irregular or insufficient. Recently, salinity and alkalinity have also become serious problems in agricultural lands of Turkey. Therefore, we have conducted researches on these environmental stresses which occur in horticultural crops. Studies that we have carried out in the last 20 years have been compiled in this review under the topics of low-temperature stress, drought stress and salt stress.

Keywords: Stress physiology, low-temperature stress, drought stress, salt stress.

ÖZET

Bazı Meyve ve Sebzelerde Stres Fizyolojisi Üzerine Çalışmalar

Türkiye coğrafi yapısı gereği farklı ekolojik bölgelere ve bu bölgelere adapte olmuş bahçe bitkilerinde geniş bir tür ve çeşit zenginliğine sahiptir. Ancak, erken ilkbahar donları ılıman ve suptropik iklim bölgelerinde önemli

* *Prof. Dr.; Uludağ Üniv., Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Bursa.*
** *Yrd. Doç. Dr.; Uludağ Üniv., Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Bursa.*
*** *Doç. Dr.; Uludağ Üniv., Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Bursa.*

miktarda ürün kaybına neden olmaktadır. Öte yandan yaz aylarında yağışların düzensiz ya da yetersiz olduğu bölgelerde, bahçe bitkilerinde kuraklık stresi ortaya çıkmaktadır. Son yıllarda ülkemizdeki tarım alanlarında tuzluluk ve alkalilik de önemli sorunlar haline gelmiştir. Bu nedenle, Türkiye'de yetiştirilmekte olan bahçe bitkilerinde ortaya çıkan bu çevre stresleri üzerine araştırmalar yapmaktayız. Son 20 yıl içinde gerçekleştirdiğimiz çalışmalar bu makalede düşük sıcaklık stresi, kuraklık stresi ve tuz stresi başlıkları altında derlenmiştir.

Anahtar sözcükler: Stres fizyolojisi, düşük sıcaklık stresi, kurak stresi, tuz stresi.

1. INTRODUCTION

An important branch of plant physiology is concerned with how plants respond to environmental conditions that deviate significantly from those that are optimal for organisms in general. As a division of physiological ecology, this field, called *stress physiology*, can contribute to our understanding of what limits plant distribution. Most research in the field, however, is concerned with how adverse environmental conditions limit agricultural production[1].

Extreme environments include those that potentially can cause stress to the organism exposed. A stress can be any environmental factor capable of eliciting from the plant a harmful chemical or causing a physical strain (change), which may be either reversible (elastic) or permanent (plastic). Such plastic strains are those caused by the stress of frost, high temperature, limited water and high salt concentrations[2,3].

Turkey has a number of different environmental conditions because of its geographical location. Therefore, a wide range of species and cultivar richness occur in terms of growing horticultural crops. Fruit and vine growing have been limited in the regions where the temperature decreases to -20°C or -25°C, especially in the Midlands and Eastern Turkey. Early spring frosts cause a considerable amount of yield loss in the regions that have temperate and subtropical climates. On the other hand, draught stress occurs in horticultural crops during summer, in the regions where precipitation is either irregular or insufficient. It is well known that regular irrigation is necessary to overcome this problem. However, the ratio of available agricultural lands has been decreasing in Turkey as well as in the World, due to increasing population and development in technology. Therefore, available water resources has been diminishing. Recently, the ratio of salinity and alkalinity problems in agricultural lands is 2% in Turkey. Moreover, this ratio is calculated as approximately 20% in Bursa[4].

Thus, we intended to investigate environmental stresses occurred in horticultural plants in Turkey. These studies are summarised in the following subsections.

2. LOW-TEMPERATURE STRESS

The stress due to low temperature is difficult to define quantitatively since threshold temperature under which a strain induced in sensitive plants depends on the tissue. However, for most plants a chilling stress can be imposed by any temperature between 10-15°C and 0°C. Only plants from tropical or subtropical regions are sensitive to this stress. On the other hand, freezing stress is a shortened way of saying "freezing low-temperature stress", because freezing is not a stress but a strain produced by low-temperature stress, *i.e.*, the plant can be exposed to temperatures below 0°C and remains unfrozen[2,3,5].

Freezing stress, in contrast to chilling stress, can occur in all plants. Because of its prevalence, freezing stress has been studied more intensively. Therefore, our studies on freezing stress aimed to determine the degree of frost resistance of some horticultural crops and physiological factors which affect this resistance.

Eriş[6], who carried out pioneering studies in Turkey, examined meristem cells of buds in grape cultivars Aris and Silvaner, which are known as frost resistant and frost sensitive cultivars, respectively. He concluded that in buds of grapes cv. Aris lipid particles were more than those of cv. Silvaner. He also concluded that this aspect showed the relationship between frost resistance and synthesis of lipids depending on the genetic characteristics of cultivars.

Subsequently, Eriş[7] determined the ability of frost resistance of some grape cultivars (*i.e.*, Çavuş, Muscat of Hamburg, Hafizali, Karagevrek and Kalecik Karası). Freezing tests were conducted between November and March, during 1980-1982. For this purpose, cuttings taken at different periods were exposed to -20°C for 24, 48, 72 and 96 hours.

Frost resistance ability was different in each cultivar. However, all the cultivars were sensitive to frost injury in November. Then frost resistance increased after December towards mid-winter. The highest frost resistance of all the cultivars occurred in February. Nevertheless, in March, there was a decline in the resistance ability of each cultivar. As the periods of cold treatment at -20°C were prolonged, the resistance of cultivars decreased and bud injury increased. The treatments, particularly of 96 hours, caused serious damage in all the cultivars.

The lowest freezing injury was observed in the Muscat of Hamburg, Kalecik Karası and Çavuş (which showed more than 50% bud break). However, there were significant differences between cultivars in terms of their frost resistance ability especially in December, January, February and March.

The most resistant cultivars were Muscat of Hamburg and Çavuş in December, Kalecik Karası in January and February, and Muscat of Hamburg in March. Moreover, Karagevrek and Hafizali were generally determined to be the most susceptible cultivars.

In another study, conducted in our Department between 1985 and 1989, frost resistance ability of peach cultivars Cardinal, Dixired, Redhaven, J.H.Hale and Halberta Giant (*i.e.*, widely grown cultivars in the Marmara Region) were determined. Moreover, seasonal changes in carbohydrates, proteins, lipids and macro and micro elements in buds and physiological relationships between these parameters and frost resistance ability of the tested cultivars were also determined[8,9,10].

Samples of one year old twigs were collected monthly from November to March and exposed to artificial freezing tests for 4, 8, 16, 24 and 48 hours at -15°C and -20°C in order to determine their frost resistance ability. There were significant differences between frost resistance ability of the tested cultivars. Redhaven was the most resistant cultivar and was followed by Dixired and J.H. Hale while Cardinal and Halberta Giant were more sensitive. The effects of artificial freezing test durations were significantly different. As the durations increased the survival percentage of buds of the tested cultivars decreased[8].

The results of biochemical analyses showed that total amount of sugars increased throughout the winter starting from November (in parallel to frost resistance ability of cultivars) and decreased in March. The amount of starch, contrarily, was high in November, low in December, January and February, and again high in March. In spite of the differences between the years and the cultivars, in general, the level of total protein increased throughout the winter starting from November, during the experimental periods. The amount of total lipid in all the cultivars increased during winter and reached to a maximum in February. Moreover, the amount of total lipid was highest in Redhaven, which was the most resistant cultivar[9].

The results of the analyses of macro and micro elements showed that the levels of nitrogen, potassium, calcium, iron and manganese were high during winter when thc frost resistance ability of the cultivars was also high. However, the level of sodium was low in winter. On the other hand phosphorus and magnesium levels were low and did not show any significant change during the experimental period. Furthermore, the amount of zinc, copper and boron were inconsistent during the experimental period. Therefore, it was concluded that there was no physiological relationship between these three secondary nutrient elements and the frost resistance ability of the tested cultivars[10].

Recently, artificial freezing tests were conducted, twice a month, between October 1989 and April 1990 in order to determine frost resistance in one-year old stems and buds of plums (cvs. Giant and Stanley) and peaches (cvs. Blake and Early Red). These tests were carried out for periods of 8, 16 and 32 hours, between -5°C and -20°C. Depending on seasonal changes, freezing temperature and duration, some differences were observed between species and cultivars in terms of the degree of freezing injury. Plums were more resistant than peaches, especially in early spring. However, occurrence of the injury was similar in tissues and organs of both species. The buds were more resistant than one-year old stems in autumn, nevertheless, it was completely opposite in spring. It was concluded due to histological observations that freezing injury increased with initiation and progress of xylem differentiation in flower buds. This could be a useful and important criterion in selection of frost resistant cultivars. It was determined that bark tissues, in autumn, and xylem, in spring, were sensitive tissues in one-year old stems. It was also concluded due to histological observations that xylem ducts were blocked by slime like materials as a result of freezing injury and, subsequently, they were attacked by micro-organisms (Eriş and Sivritepe, unpublished data).

3. DROUGHT STRESS

The interest in the effect of drought stress on plants results mainly from the need to better understand the problems to which economically important crop plants are exposed when water is a limiting factor. Kramer[11] suggests that the world-wide losses in yield caused by water shortage are greater than those caused by all other causes together.

The water balance of the plant should well be established in order to avoid drought stress. Maintenance of the water balance of the plant depends on morphological, physiological and genetic characteristics of plants and ecological conditions in which plants are grown. Stomata has the most important role in this phenomenon since 90% of water loss originate from them[12]. Thus, the objective of our initial studies was to increase stomatal resistance by avoiding water loss.

In a study conducted by Eriş[13], the effects of some plant growth regulators (GA, Ethrel, B-9 and CCC) at different levels (0, 100, 500 and 1000 ppm) on stomatal resistance of leaves in tomato and pepper seedlings were determined. GA_3 treatments reduced stomatal resistance of leaves in tomato and pepper seedlings. In tomato leaves, 1000 ppm and in pepper leaves 100 ppm were the most effective doses. Ethrel treatments increased stomatal resistance of leaves of both plants. In tomato leaves, all levels of Ethrel and in pepper leaves 1000 ppm were the most effective doses. The B-9 treatments reduced stomatal resistance of leaves in both plants. 100 ppm in tomato leaves and 500 ppm in pepper leaves were the most effective doses. Especially, 500 and 1000 ppm CCC treatments increased stomatal resistance of leaves in tomato seedlings. On the other hand, all concentrations reduced leaf stomatal resistance in pepper seedlings.

In another experiment, Eriş *et al.*[14] investigated the effects of four different plant growth regulators (GA_3, CEPA, B-9 and CCC) on the number of total stomata and the rate of closed/open stomata in the upper epidermis of the leaves of tomato and bean seedlings. GA_3, B-9 and CCC increased the number of total stomata in tomato and bean leaves; but CEPA applications, except with tomato, had no effect on bean leaves. None of these growth regulators changed the rate of closed stomata in tomato. Only CCC increased the rate of closed stomata in bean (from 26.9% to 35.5%), but the other regulators had also no significant effect on the rate of closed stomata in bean leaves.

Eriş[15] also investigated the effects of exogenous treatments of salicylic acid on stomatal resistance in seedlings of some pepper cultivars. Four doses of salicylic acid treatments (100, 200, 400 and 800 ppm) had, generally, similar effects on stomatal resistance of different pepper cultivars. Especially, 400 and 800 ppm treatments significantly increased stomatal resistance of leaves. These results were obtained 24 hours after treatments and also from the average results of continuous measurements of 5 days after treatments.

Eriş and Soylu[16], investigated the possible effect of size and density of stomata on drought tolerance in 15 Turkish grape cultivars. Number of stomata per mm^2 varied from 129±18 to 254±10, stomatal length ranged from 22.6±2.6 µm to 28.3±4.3 µm, and stomatal width from 13.6±2.2 µm to 18.6±3.2 µm among the cultivars. The minimum and maximum number of stomata were observed in Balbal and Pembe Gemre, respectively. The minimum and maximum size of stomata were observed in Erenköy Beyazı and Müşküle, respectively. There were significant differences between the cultivars in terms of drought tolerance. The most sensitive cultivars were Çavuş, Amasya, Tarsus Beyazı and Sultani Çekirdeksiz while the most tolerant cultivars were Yapıncak and Balbal. However, the relationship between size and density of stomata and drought tolerance remained uncertain due to conflicting results obtained from the cultivars.

Recently, in a research conducted in our Department between 1990 and 1992[17], the aim was to determine the drought resistance ability of Early Red, Red Haven, J.H. Hale and Rio-Oso-Gem peach cultivars grafted on seedling, GF-305 and Nemaguard rootstocks and nectarine cultivars Independence, Nectared-4 and Nectared-8 grafted on Nemaguard rootstocks. Moreover, the physiological and morphological changes of the cultivars as well as their resistance to various durations of different watering regimes (100%, 75%, 50% and 25% of available soil water) were observed. The differences between drought resistance ability of all cultivar/rootstock combinations were significant. GF-305 was found to be the most sensitive rootstock to water deficit, and it was followed by seedling and Nemaguard rootstocks. As far as the cultivars were concerned, Early Red seemed to be the most sensitive followed by Red Haven, Rio-Oso-Gem and J.H.Hale. In nectarines, Nectared-

8 was the most sensitive cultivar followed by Independence and Nectared-4 cultivars. In general, when the amount of water given to the plants decreased the water potential, relative water content and chlorophyll-a, b and total chlorophyll contents and total starch content of leaf also decreased. However, total sugar and ABA contents in the leaves increased. Growth of the shoots and trunk diameter was retarded with the decrease in watering levels. Moreover, the growth of the plants was inhibited at the lowest watering level. Consequently, leaf relative water content, leaf water potential and chlorophyll content were found to be more important than the total sugar and starch contents on the physiology of drought resistance[17].

4. SALT STRESS

According to Levitt[3], "if the salt concentration is high enough to lower the water potential appreciable (0.5-1.0 bar), the stress will be called as salt stress". Salt stress may have primary and secondary effects. Primary salt injuries may include direct, specific toxic effects as well as indirect effects, such as metabolic disturbances and inhibition of growth and development. Secondary salt effects include nutrient deficiency and osmotic dehydration. The estimation of the contribution of the primary and secondary effects to salt injury is still an open question.

Excess salt, usually NaCl, is the most widespread chemical condition inhibiting plant growth in nature[18]. The major efforts to circumvent salinity in the past have been directed toward soil reclamation and water desalination-practices that are becoming increasingly expensive. Therefore, these efforts must coincide with the measures to improve salt resistance of crops through genetic modification[19]. In some species, the diversity of salt resistance among cultivars seems quite extensive, and conventional breeding techniques are being used to improve their salt resistance[20]. In many species with less diversified nature for salt resistance, promising approaches would be either to use variation existing in wild relatives or to use tissue culture techniques for selection of resistant plants as well as mutations for salt resistance.

Between 1992 and 1995, a research was carried out in our Department by Eriş and Sivritepe to develop a convenient method for grapes in determining salt resistance at an early stage. Salt resistance tests were conducted on 5 BB, 41 B and 1613 grape rootstocks and in Çavuş, Müşküle and Sultani Çekirdeksiz grape cultivars under *in vitro* and greenhouse conditions[21].

Plant materials used in *in vitro* salt resistance tests were propagated by the axillary bud culture method[22]. Single-node shoots were subjected to five different NaCl concentrations (0.00, 0.25, 0.50, 0.75 and 1.00%) in MS + 5 μM BAP medium for two different periods (4 and 8 weeks). Proliferation ratio, weight, shoot length and number, 3-node shoots, leaf number of shoots, total chlorophyll content and viability of explants decreased due to the increase in NaCl concentration and treatment period. Moreover, it was determined that salt treatments caused necroses in explants, and the severity of the injury varied depending on the NaCl concentration and treatment period.

Plant materials used in salt resistance tests under greenhouse conditions were obtained by sprouting single nodal cuttings with axillary buds, in growth containers filled with perlite. When their shoots reached to the single node stage, the cuttings were subjected to salt for 4 weeks by irrigating them with MS solutions containing similar NaCl concentrations used in *in vitro* experiments. Shoot weight and length, root weight, node and leaf numbers, total chlorophyll contents and viability of cuttings decreased, and the severity of injury increased due to the increase in NaCl concentrations. Furthermore, salt treatments inhibited root formation in cuttings and root growth. Salt treatments caused Na accumulation in all organs (*i.e.,* roots, shoots, pedicels and lamina) of the plant. Moreover, K:Na ratio decreased whereas Na:Ca ratio increased. These effects were strengthened with the increase in NaCl concentrations.

Having determined the injurious effects of salinity in grape rootstocks and cultivars after NaCl treatments, conducted under *in vitro* and greenhouse conditions, salt resistance in grapes and differences between rootstocks and cultivars were also determined by the use of obtained data. Beside the percentage viability, tolerance ratio and tolerance index were calculated on the bases of explant weight (which was used to designate growth) and total chlorophyll (which was used to designate metabolic disturbance) to determine salt resistance of grape rootstocks and cultivars during *in vitro* salt treatments. However, in greenhouse salt resistance tests, tolerance ratio and tolerance index calculated on the basis of root weight (which was used to designate root growth), Na contents of different organs and the ability of maintaining K:Na and Na:Ca balances were used in addition to the above parameters.

After the evaluation of these criteria, it was concluded that the differences in salt resistance of grape rootstocks and cultivars were similar under both conditions (i.e., *in vitro* and greenhouse experiments). The most resistant grape rootstock to salt treatments was 1613 and was followed by 5 BB and 41 B. Furthermore, the most resistant grape cultivar to salt treatments was Çavuş, and was followed by Sultani Çekirdeksiz. Nevertheless, Müşküle was the most sensitive cultivar to salinity. It was also concluded that under greenhouse conditions, the tolerance limits regarding NaCl concentration were different from *in vitro* conditions.

It was determined that salt resistant grape rootstocks and cultivars could relatively maintain their growth rates and could avoid metabolic disturbances such as chlorophyll deficiency. Moreover, grape rootstock 1613 and cultivar Çavuş, which were more resistant to salinity than the other rootstock and cultivars, took up less Na through their roots and excluded from their leaves and, therefore, avoided salt injury. Their ability to maintain ion balances (Na:Ca and K:Na) was determined to be an important factor in salt resistance. Thus, grapes which showed salt resistance had higher ratio of K:Na in their lamina and lower ratio of Na:Ca in their roots compared with the salt sensitive ones.

The results of the present study showed the advantages and usefulness of salt tests conducted by the use of axillary bud culture under *in vitro* conditions. Besides using parameters which showed growth and metabolic disturbances, investigation of ion accumulation and ion balances should be necessary in determination of salt resistance. The utilisation of tolerance ratio and tolerance index was found to be a good evaluation method for classification of different rootstocks and cultivars.

In another study, possibilities of using NaCl priming were investigated to increase salt tolerance of melon seeds (cvs. Hasanbey and Kırkagaç) during germination. Priming treatments of both melon cultivars were conducted for 3 days at 20°C by the use of various concentrations (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%) of NaCl. The NaCl concentration of 1.0%, which did not show any difference compared with control due to total germination and mean germination time parameters, was determined as the optimum dose for priming treatments conducted in melon seeds. Then, seeds primed with 1.0% NaCl were taken to germination tests with different NaCl concentrations (0.0, 0.25, 0.50, 0.75 and 1.00%). Beside the results of total germination and mean germination time, the results of tolerance ratio and tolerance index clearly showed that priming treatments affected the increase of salt tolerance in both melon cultivars. Furthermore, it was concluded that cv. Kırkagaç was more tolerant to salinity than cv. Hasanbey[23].

In the subsequent studies, melon seeds (cvs. Hasanbey and Kırkagaç) primed (P) with 1.0% NaCl were sown in peat medium and irrigated with different NaCl solutions (0.0, 0.25, 0.50, 0.75 and 1.00%) for 4 weeks to examine their response to salinity. Physical analyses such as total emergence, dry weight and fresh weight were carried out. For each melon cultivar, tolerance index and tolerance ratio were determined on the basis of fresh weight. Moreover, chemical analyses such as total sugar, proline, accumulation of Na, Ca and K were also carried out. K:Na and Na:Ca ratios were calculated to clearly show the ion metabolism of melon seedlings during salinity stress. The results suggested that in both melon cultivars seedlings derived from primed seeds had higher adaptation capacity to salinity. Furthermore, the results of this study revealed that accumulation of sugar and proline was higher in melon seedlings derived from primed seeds than those from non-primed seeds. Therefore, the higher adaptation capacity of seedlings in primed groups to salinity could be due to osmoregulation induced by physiological changes. On the other hand, NaCl priming induced avoidance of melon seedlings from toxic and nutrient deficiency effects of salinity. In conclusion, these studies showed for the first time that NaCl priming of melon seeds could be used to increase salt tolerance of seedlings. Although cv. Kırkağaç was more tolerant to salinity than cv. Hasanbey, beneficial effects of NaCl priming was observed in both cultivars[24,25].

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