Diagnostics of plum resistance to the combined effects of drought and hyperthermia

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Abstract. The article considers the possibility of diagnosing the stability of fruit crops under the combined action of hyperthermia and drought. The objects of the study were isolated leaves of _Prúnus doméstica_ L. of FSBSI VNIISPK breeding varieties. The purpose of the work was to assess the resistance of plum varieties to the combined effects of drought and hyperthermia on the basis of physiological and biochemical resistance markers. The products of peroxidation of membrane lipids, free proline, hydrogen peroxide and catalase activity were determined. It is shown that after the action of the stresses used in the varieties Nezhenka and Orlovsky souvenir, the accumulation of malondialdehyde and lipid hydroperoxides was significantly lower by 23.0...24.6% and 15.6...16.3% than in the varieties Skoroplodnaya and Krasa Orlovschiny by 42.1...52.3% and 36.6...41.2%, respectively. It was shown that against the background of the action of stressors, the varieties Nezhenka and Skoroplodnaya were characterized by a high degree of intensification of the antioxidant enzyme catalase and a moderate increase in hydrogen peroxide in the leaves. Thus, a slight increase in the intensity of lipid peroxidation in the Nezhenka and Orlovsky souvenir varieties in comparison with the Krasa Orlovschiny and Skoroplodnaya indicates the presence of physiological and biochemical resistance to the action of the stress factors used.

1. Introduction
Fruit plants, as a result of a long-term life cycle, are exposed to a different impact not only in terms of strength, but also in terms of duration of the influence of many stress types. In addition, the natural ability to withstand adverse environmental factors may decrease in old varieties. In this regard, in the conditions of climate change, plant breeding faces the task of creating new varieties characterized not only by high yields, but also having resistance to stress factors, as well as to their frequent alternation. To implement such a breeding process, it is necessary to identify sources of resistance to adverse environmental factors with their further inclusion in the breeding process. One of these methods is the modeling of stress factors, followed by the identification and determination of physiological and biochemical markers of resistance in plant tissues. These include indicators of the antioxidant system activity, the intensity of membrane lipid peroxidation (MLP), as well as the level of reactive oxygen species (ROS). Thus, a number of studies have shown that under the influence of stress factors in plant tissues, there is an increase in MLP intensity, activity of antioxidant components, as well as an increase in the formation of ROS [1,2,3,4]. In this regard, the purpose of the work was to assess the resistance of plum varieties to the combined effects of drought and hyperthermia on the basis of physiological and biochemical resistance markers.
2. Materials and methods
The work was carried out on the basis of the Laboratory of physiology of resistance of fruit plants of the FSBSI VNIISPK. The object of the study was the varieties *Prunus domestica* L. of VNIISPK breeding: Orlovsky souvenir, Nezhenka, Krasa Orlovschiny, Skoroplodnaya. To simulate drought conditions against the background of hyperthermia, isolated plant leaves were placed in laboratory glasses with a 25% solution of polyethylene glycol in a Binder dry-air thermostat (Germany) at a temperature of 50°C. Control - leaves in glasses with water at a temperature of 23°C. The exposure time is 1 hour.

The intensity of lipid peroxidation was determined on the basis of quantitative analysis of the content of lipid hydroperoxides and malonic dialdehyde based on reactions with ammonium rhodanide and thiobarbituric acid, respectively. \( \lambda = 520 \text{nm} \) was used for malondialdehyde and \( \lambda = 480 \text{nm} \) for lipid hydroperoxides. The amount of hydrogen peroxide, as one of the representatives of reactive oxygen species, was found using titanium tetrachloride at \( \lambda = 420 \text{nm} \) [5]. The level of free proline was found on the basis of a qualitative reaction with a ninhydrin reagent at \( \lambda = 520 \text{nm} \) [6]. The catalase activity was judged by the amount of molecular oxygen released during the decomposition of \( \text{H}_2\text{O}_2 \) [5]. The analyses were performed in five-fold repetition, the determinations were performed on a BioRad SmartSpec Plus spectrophotometer (USA). The data is statistically processed using the Excel MS Office software package.

3. Results and Discussion
It is shown that after the end of the action of the stress factors used in the leaf tissues of annual plum shoots, an intensification of oxidative processes and, in particular, peroxidation of membrane lipids was observed. This is evidenced by a significant increase in the accumulation of malondialdehyde in tissues. This substance is the final product of lipoperoxidation of membranes and is one of the key indicators that allows to judge the structural and functional integrity of biological membranes after oxidative exposure. According to the results obtained, after the end of the stressors, the greatest structural and functional damage to cell membranes was received by plum varieties Krasa Orlovschiny and Skoroplodnaya. In the studied leaves of these varieties, the amount of MDA increased by 52.3% and 42.1%, respectively, against their controls under normal conditions (Figure 1). On the contrary, in the varieties Orlovsky souvenir and Nezhenka, the amount of MDA increased slightly by 24.6% and 23.0%.

![Figure 1](image_url)

**Figure 1.** The content of malondialdehyde (MDA) in the leaf tissue of varieties of bullace plum after the action of hyperthermia and drought.
1 - Orlovsky souvenir, 2 - Nezhenka, 3 - Krasa Orlovschiny, 4 - Skoroplodnaya

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It is known that with the presence of ferrous ions, hydrogen peroxide as a ROS representative can decompose to form a hydroxyl radical (Fenton reaction), which is extremely active and destroys almost any molecule it encounters [7]. The initiation of a chain reaction (MLP) begins with the introduction of a free radical – a hydroxyl radical - into the lipid layer of membranes or lipoproteins. With polyunsaturated fatty acids of the membranes, the hydroxyl radical forms a lipid radical [8, 9]. Subsequently, with molecular oxygen, the lipid radical forms a lipoperoxide radical, which, when interacting with phospholipids, forms lipid hydroperoxides and a new lipid radical. The alternation of the last two reactions is a chain reaction of peroxidation (peroxidation) of lipids [10, 11]. At the places of attachment of peroxide radicals, fatty acids are torn into fragments, on the edges of which aldehyde groups with high reactivity are located. If the rupture occurred on both sides, secondary products are formed, which include oxygen-containing compounds: aldehydes and dialdehydes, in particular, malondialdehyde [12, 13, 14]. As a result, the quantitative determination of hydroperoxides makes it possible to directly judge the intensity of free radical processes.

It was shown that varieties with a high accumulation of malondialdehyde also had a high level of lipid hydroperoxides. Thus, under the influence of drought together with hyperthermia, the content of lipid hydroperoxides in the varieties Krasa Orlovschina and Skoropladnaya increased by 41.2% and 36.6% (Figure 2).

![Figure 2](image-url)

**Figure 2.** The content of lipid hydroperoxides (LH) in the leaf tissue of bullace plum varieties after the action of hyperthermia and drought.
1 - Orlovsky souvenir, 2 - Nezhenka, 3 - Krasa Orlovschina, 4 - Skoropladnaya

Along with this, in the Orlovsky souvenir and Nezhenka varieties, the level of LH increased only by 15.6% and 16.3% against their controls. The correlation coefficient between LH and MDA of the Orlovsky souvenir and Nezhenka varieties was 0.45, and that of the Skoropladnaya and Krasa Orlovschina varieties was 0.93. Apparently, this may indicate a more effective neutralization of reactive oxygen species and lipid hydroperoxides in the varieties Skoropladnaya and Nezhenka by the antioxidant protection system of leaf tissue or by their initially insignificant formation. Thus, in the varieties Krasa Orlovschina and Skoropladnaya, the content of lipid hydroperoxides under the influence of drought together with hyperthermia increased by 41.2% and 36.6%, and in the Orlovsky souvenir and Nezhenka - by 15.6% and 16.3%. At the same time, the correlation coefficient between the accumulation of malondialdehyde and lipid hydroperoxides in the varieties Krasa Orlovschina and Skoropladnaya was 0.93, whereas in the Orlovsky souvenir and Nezhenka - 0.45, which may indicate partial neutralization of lipid hydroperoxides in resistant varieties by an antioxidant protection system.
To determine the work of the antioxidant defense system, the content of the low-molecular proline antioxidant and catalase activity were determined. As a result, it is shown that in the varieties Orlovsky souvenir and Nezhenka, after the action of hyperthermia and drought, the intensity of free proline accumulation practically did not occur, whereas in the varieties Krasa Orlovschiny and Skoroplodnaya increased by 26.5 ...31.5% (Figure 3). According to S. Alia et al. [15] the proline content in the plant under stress increases against the background of insufficient work of antioxidant enzymes for the purpose of direct "quenching" of reactive oxygen species and free radicals.

![Figure 3. The content of free proline in the leaf tissue of bullace plum varieties after the action of hyperthermia and drought.](image)

The antioxidant enzyme - catalase, utilizing reactive oxygen species on the example of hydrogen peroxide, showed an ambiguous intensity of work in the studied varieties. Thus, in the varieties Orlovsky souvenir and Nezhenka, the activity of the enzyme after the action of stressors increased by 2.0...2.2 times, and in the Krasa Orlovschiny and Skoroplodnaya - by 1.4...1.5 times (Figure 4).

![Figure 4. Catalase activity in the leaf tissue of bullace plum varieties after the action of hyperthermia and drought.](image)
Correlation analysis showed that under stress-free conditions there is no interrelation between catalase activity and free proline ($r = 0.07$), however, under the influence of stressors, the correlation coefficient is $r = -0.48$. This is partly proved by the fact that proline accumulates to a greater extent in those plants where there is insufficient enzyme activity.

When determining hydrogen peroxide as one of the representatives of reactive oxygen species after the action of stressors, its significant accumulation in varieties with a high level of peroxidation of membrane lipids, intensive accumulation of free proline and insufficient enzyme activity is shown. Thus, the varieties Krasa Orlovschiny and Skoroplodnaya, when exiting stress, contained 89.1 and 91.0% more hydrogen peroxide in leaf tissue than their leaves under normal temperature conditions (Figure 5). At the same time, the Orlovsky souvenir and Nezhenka increased the level of hydrogen peroxide by 38.5 and 54.8% compared to the corresponding samples under stress-free conditions. Apparently, due to higher catalase activity and low level of hydrogen peroxide formation, the varieties Orlovsky souvenir and Nezhenka had minor structural and functional damage to cell membranes, as evidenced by a relatively small accumulation of malondialdehyde and hydroperoxides when plants exiting stress.

![Figure 5. The content of hydrogen peroxide in the leaf tissue of bullace plum varieties after the action of hyperthermia and drought.](image)

1 - Orlovsky souvenir, 2 - Nezhenka, 3 - Krasa Orlovschiny, 4 - Skoroplodnaya

4. Conclusion
When modeling a drought with hyperthermia, the varieties Nezhenka and Orlovsky souvenir had the greatest resistance to the action of the stressors used. In the leaves of these varieties, after the action of the stressors used, there was a slight accumulation of malondialdehyde, the final product of lipoperoxidation, against the background of a high degree of intensification of the antioxidant enzyme catalase and a moderate increase in hydrogen peroxide in the leaves, in contrast, the varieties Krasa Orlovschiny and Skoroplodnaya were characterized by a high degree of development of membrane lipid peroxidation, insufficient enzyme activity, accumulation of free proline and a high level of formation of reactive oxygen species.

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