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Methods of determining the drought resistance of plants

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Abstract.
This article presents methods for determining the drought resistance of plants at different stages of development. To determine drought resistance at the early stages of plant development, when creating breeding material, it is necessary to use the most accessible and common laboratory methods, which include modeling moisture deficit in solutions with increased osmotic pressure, which consists in the ability of germinated seeds of a variety to use low moisture reserves in the soil and predict resistance genotypes to soil moisture deficiency in the early stages of ontogenesis. At various stages of plant vegetation, recently, indirect assessment of drought resistance using physiological methods is becoming increasingly common. The most informative are methods of studying the water regime of leaves, namely: determination of tissue hydration, water deficit and water-holding capacity of leaves. Leaf water loss reflects the water status of plants and is key to their survival in drought conditions. The indicator of water-holding capacity characterizes the plant's ability to resist dehydration for a more or less long time, the length of which depends on its level. The water status of the plant is determined by the water deficit, which is an important indicator of drought resistance. Watering plants indicates that they are supplied with water, which is necessary for the flow of biochemical reactions (that is, for vital activity) and is one of the important indicators of the water regime of plants. The water

content in the leaves indicates the attitude of the plants to the lack of moisture. To differentiate genotypes by drought resistance, various selection indices are used, based on the productivity of plants in optimal and stressful conditions for the selection of drought-resistant genotypes.

Keywords:
drought resistance
osmotic stress
water deficit
water-holding capacity
mathematical indices

AGROTECHNOLOGIES AND AGRICULTURAL INDUSTRY

According to forecasts, global climate change will lead to an increase in temperature, a change in the geographic structure of precipitation and, in the future, an increase in the frequency of extreme climatic events, an increase in the intensity and duration of droughts [1, 2, 3]. Drought is the most common environmental problem, with nearly 25% of the world's agricultural land restricted to growing crops. The combination of physical and environmental factors causes stress in plants and reduces yield [4, 5]. Currently, arid and semi-arid regions account for about 30% of the total area of the world, where about 20% of the world's population lives [6, 8]. These areas are expanding, and it is expected that they will continue to increase and the share of the earth's surface under conditions of severe drought will increase from 1% at present to 30% by the end of the 21st century [8, 9]. Drought can have a serious impact on ecosystems, agriculture, water resources, energy supply and general human well-being as a whole. Fertile lands are becoming scarce. And the competition for such lands is intensifying, which is why the demand for food is growing [10, 11, 12].

It is known that the lack of water in the soil causes much more damage to crop production than all other stress factors combined [13, 14, 15]. The harmful effect of drought consists, first of all, in dehydration and disruption of metabolic processes in plants, which leads to the breakdown of proteins, a change in the colloidal and chemical state of the cell cytoplasm and, as a result, to a decrease in the amount of organic matter accumulated by plants, less intense accumulation of dry matter [16 , 17, 18]. In general, the reaction of plants to drought is complex, and plants under the influence of drought show different adaptive reactions at the morphological, physiological and molecular levels with large genotypic variations, and the type of reaction is determined by the intensity of the drought and its duration [19, 20]. In response to stress, in order to avoid an extreme period, plants can adjust the speed of seasonal development and maturation, reduce the area of the leaf surface [21, 22].

O.O. Zhuchenko believes that the adaptation of plants to a lack of water is manifested in their xeromorphic, greater endurance to dehydration, that is, in the presence of mechanisms that allow reducing water loss by plants [23]. For example, in transgenic alfalfa plants with an increased wax coating of the leaf cuticle, transpiration is reduced, water is better retained in the plant, and therefore slow wilting occurs after the cessation of irrigation and faster and better recovery after the resumption of irrigation [24, 25]. Reducing water losses can be achieved due to the compact shape of the crown, that is, the habitus or architecture of the plant, small bushiness, weak foliage [26, 27]. Water stress leads to inhibition of plant growth processes, including and root system, therefore, an important feature of drought resistance is the strength of the root system, its mass, absorption capacity, and the depth of its penetration. According to M.O. Maksimova, O.O. Zhuchenko, A.P. Orlyuk and others. drought resistance is the ability of plants due to signs or properties to withstand adverse growing conditions and not reduce the yield and is defined as a percentage of productivity reduction: the smaller the yield reduction, the higher the drought resistance [28, 29, 30]. The nature of the reaction of plants to water stress is determined by the whole complex of factors: physiological state, biological features of plants and others. Drought-resistant can be called plants that are able to adapt to the effects of drought and carry out normal growth, development and reproduction in these conditions, tolerate temporary dehydration with the least reduction in growth processes and productivity. This happens due to the presence of properties that arise in the process of evolution under the influence of living conditions and natural selection [31, 32, 33].

Field and laboratory methods are used to diagnose drought resistance of plants. In field conditions, varieties and species of plants that are compared, grown in arid areas and that reduce yields to a lesser extent, are considered more drought-resistant. It is possible to assess varieties for drought resistance in established special dryers and dry-warping installations, where it is possible to create soil and atmospheric drought for plants in any period of their vegetation [34, 35, 36].

The selection of naturally resistant and better adapted to stress conditions genotypes among different varieties is a promising prospect and the cheapest, most effective method of plant selection. Identifying and creating drought-resistant genotypes is one of the main tasks of breeding programs, but creating high-yielding varieties and realizing their yield potential in arid conditions is an extremely difficult task for breeders [37].

To assess drought resistance, one of the most available and common laboratory methods is the method with simulation of moisture deficit in solutions with increased osmotic pressure. A greater number of germinated seeds indicates the variety's ability to use low moisture reserves in the soil and characterizes its drought resistance. Thus, at the early stages of ontogenesis, it is possible to predict the resistance of genotypes to soil moisture deficiency [38] and to distinguish populations that show tolerance to stress in the early phase of growth [40]. The use of different osmotic materials is considered one of the best methods of studying the effect of osmotic stress on seed germination at different stages of selection [40]. It is possible to predict the level of drought tolerance of agricultural crops when germinating seeds using sucrose [41], polyethylene glycol (PEG) [42]. This method has important advantages: simplicity and availability of implementation, low labor intensity and independence from weather conditions, which allows conducting research all year round. Seeds cultivated in osmotic solutions simulate a lack of moisture due to the fact that sucrose extracts water from living cells. Drought-resistant plants have a high water-holding capacity. Therefore, the greater the number of seeds germinated on the sucrose solution, the greater the drought resistance of the plant [43, 44].

The percentage of germinated seeds (*P*) was calculated according to the formula:

$$P = 100\% \times \frac{a}{b} \quad (1)$$

where a – is the average number of seeds germinated in the sucrose solution, b – is the average number of seeds germinated in the control (on distilled water).

The level of drought resistance of the sample was determined by the number of seeds germinated in sucrose solutions [38, 41].

In addition to the specified properties, take into account the peculiarity of the growth of seedlings. The method of determining seed growth strength was used to evaluate the character of alfalfa seed germination by root and hypocotyl length in different sucrose solutions according to a 5-point system [45].

Seedling growth index (VI) was determined by the formula:

$$VI = RL + \frac{SL \times GP}{100} \quad (2)$$

where RL – is the length of the germinal root; SL – hypocotyl length; GP – seed germination [46].

The ability of seeds to germinate well in conditions of "physiological drought" indicates both the ability of the plant to germinate with a small amount of moisture, and the high suction power of the seeds, which makes it possible to absorb more water from solutions. This approach makes it possible to judge the features of seed germination in the absence of moisture and to control the process of plant resistance to water deficit in the early stages of ontogenesis. It was established that species and varieties differ in seed similarity under stressful conditions [4, 42, 47].

The assessment of functional disorders under water and temperature stress is important for establishing the adaptive capacity and resistance of plants to stresses and for predicting the limits of tolerance to drought and overheating. Therefore, selection for stress resistance should be based on the use of morphophysiological traits characterizing high plasticity and productivity of plants in adverse conditions. For this, it is necessary to study the influence of water and temperature stress on the course of physiological processes in different periods of plant growth and development [48]. Knowledge of these indicators makes it possible to assess the degree of adaptation of plants to a lack of moisture [49].

In this regard, the methods of diagnosing the functional state of plants, which most accurately reflect their stability, become important. Therefore, in order to speed up the selection process, recently, more and more frequently resorting to indirect assessment of drought resistance using physiological methods.

The most informative are the methods of studying the water regime of leaves: determination of tissue watering, water deficit and water-holding capacity of leaves. Changes in the water regime can cause important protective and adaptive reactions of plants to environmental conditions. As a rule, under stressful conditions, the watering of plant tissues is significantly reduced and water is redistributed in the cell. At the same time, the amount of water that is difficult to extract increases and the amount of weakly bound water decreases sharply. As a result, the mobility of water and the activity of metabolic processes decrease, but the water-holding capacity of tissues and the resistance of plants to extreme conditions increase [50]. Therefore, resistance to drought depends on the unequal ability of cells to resist the extraction of water, hence the difference in the resistance of plants to wilting of leaves. Therefore, it is possible to diagnose the drought resistance of plants by the loss of water from cut leaves and twigs. Leaf water loss reflects the water status of plants, and it is the key to plant survival under drought stress conditions [51, 52]. Thus, the water-holding capacity characterizes the ability of plants to accumulate and retain moisture in the plant for a more or less long time. The slower a plant loses water, the higher its water-holding capacity and, therefore, it can withstand dehydration longer, and precisely this indicator characterizes the ability to resist dehydration and can characterize the adaptability of plants [53]. Thus, the water-holding capacity characterizes the ability of plants to accumulate and retain moisture in the plant for a more or less long time.

The water-holding capacity of leaves was calculated according to [54]. The leaves (30 pieces) were weighed, then placed on grids in a thermostat with a constant temperature (25 °C) and air humidity. After 2 and 8 hours, repeated weighing was carried out to determine water loss. Water loss during wilting is related to water-holding capacity, that is, the ability of leaf tissues to hold a certain amount of water.

The water-holding capacity was determined by the formula:

$$W_c = 100 \times \frac{M_2}{M_1} \quad (3)$$

where W_c – is the water-holding capacity, or water loss, %; M_1 – mass of leaves before wilting, g; M_2 – is the mass of leaves after a certain period of time, g.

An important indicator of drought resistance is the water deficit, which determines the water status of the plant. It is understood as the ratio of the amount of missing water required for complete saturation of cells to its total content at full tissue saturation, expressed as a percentage. A water deficit occurs in a plant during a hot day and is fully or partially replenished during the night, i.e. a residual water deficit indicates a violation of the balance between water intake and consumption. This indicator in plants leads, first of all, to a decrease in the content of free water, a simultaneous increase in the concentration of cell juice, as a result of which profound changes occur in the cytoplasm, its viscosity increases, membrane permeability increases, and cells lose their ability to absorb nutrients [55].

The water deficit of leaves was determined according to the method of J. Čatský [56]. After weighing, alfalfa leaves (30 pcs.) were placed in flasks with water for saturation. The flasks were placed in a vessel of water and covered with the same vessel to create a wet chamber. After 24-hour saturation, the leaves were dried with filter paper and weighed. The water deficit in the leaf (the ratio of the amount of incoming water to the total water content in the state of full saturation, expressed as a percentage) was calculated using the formula:

$$WD = 100 \times \frac{M_2 - M_1}{M_2 - M_3} \quad (4)$$

where WD – is water deficit, %; M_1 – mass of leaves before 24-hour saturation, g; M_2 – mass of leaves after 24-hour saturation, g; M_3 – dry mass of leaves, g.

The assessment of functional disorders under water and temperature stress is important for establishing the adaptive capacity and resistance of plants to arid conditions and for predicting the limits of tolerance to drought and overheating. Therefore, selection for stress resistance should be based on the use of morpho-physiological traits characterizing high plasticity and productivity of plants in adverse conditions. For this, it is necessary to study the influence of water and temperature stress on the course of physiological processes in different periods of plant growth and development [48].

The response of plants to water deficit can help reveal the genetic and physiological mechanisms that determine drought resistance, and in irrigated agriculture will contribute to increasing the efficiency of water use, but information on the corresponding adaptive characteristics is limited [57].

To differentiate genotypes for drought resistance, various mathematical indices are used, based on the productivity of plants in optimal and stressful conditions [29, 46] for the selection of drought-resistant genotypes [58, 59].

Productivity and drought resistance were determined using various indices:

$$\mathrm{MP} = \frac{Yp+Ys}{2} \qquad (5)$$

Rosielle and Hamblin (1981) [60]
where MP – the average yield, Yp – the yield under optimal conditions, Ys – the yield under stressful conditions.

$$D = 1 - \frac{\overline{Ys}}{\overline{Yp}} \qquad (6)$$

Blum (1988) [61]
where D – drought intensity, $\overline{Yp}$ *– average yield of all varieties under optimal conditions,* $\overline{Ys}$ *– average yield of all varieties under stressful conditions.*

The stress susceptibility index (SSI) is a good indicator for identifying high-yielding genotypes that are also highly resistant to stress. As a rule, a lower level of SSI indicates less variation in the yield of a variety under stress and under optimal conditions.

$$SSI = \frac{1-\frac{Ys}{Yp}}{1-\frac{\overline{Ys}}{\overline{Yp}}} \quad (7)$$

Fisher and Maurer, (1978) [62]
where SSI – drought susceptibility index.

$$TOL = Yp - Ys \quad (8)$$

Rosielle and Hamblin (1981) [60]
where TOL – drought tolerance index.

$$YSI = \frac{Ys}{Yp} \quad (9)$$

Bouslama and Schapaugh (1984) [63]
where YSI – crop stability index.

$$YI = 100 \times \frac{Ys}{\overline{Ys}} \quad (10)$$

Gavuzzi et al. (1997) [64]; Lin et al. (1986) [65]
where YI – yield index.

$$STI = \frac{Ys \times Yp}{\overline{Yp}^2} \quad (11)$$

Fernandez (1992) [66]
where STI – stress tolerance index.

$$GMP = \sqrt{Ys \times Yp} \quad (12)$$

Fernandez (1992) [66]; Kristin et al. (1997) [67]
where GMP – average geometric (proportional) yield.

$$RDI = \frac{\frac{Ys}{Yp}}{\frac{\overline{Ys}}{\overline{Yp}}} \quad (13)$$

Fischer and Maurer (1978) [62]
where RDI – index of relative resistance to drought.

$$DI = \frac{Ys \times \left(\frac{Ys}{Yp}\right)}{\overline{Ys}} \quad (14)$$

Blum (1988) [61]; Lan (1998) [68]
where DI – drought resistance index.

$$SSPI = 100 \times \frac{Yp - Ys}{2 \times \overline{Yp}} \quad (15)$$

Moosavi et al. (2007) [69]
where SSPI – index of susceptibility to stress.

$$M_1STI = STI \times \left(\frac{Yp}{\overline{\overline{Yp}}}\right)^2 \quad (16)$$

$$M_2STI = STI \times \left(\frac{Ys}{\overline{\overline{Ys}}}\right)^2 \quad (17)$$

$$MSTI = M_1STI \times M_2STI \quad (18)$$

Farshadfar and Sutka (2002) [70]
where M_1STI, M_2STI – modified stress tolerance indices.

$$ATI = \frac{Yp - Ys}{\frac{\overline{Yp}}{\overline{Ys}}} \times \sqrt{Yp \times Ys} \quad (19)$$

Moosavi et al. (2007) [69]
where ATI – index of abiotic tolerance.

$$HMP = 2 \times \frac{Yp \times Ys}{Yp + Ys} \quad (20)$$

Kristin et al. (1997) [67]; Chakherchaman et al. (2009) [71]; Jafari et al. (2009) [72]
where *HMP* – harmonic mean performance.

$$ISR = \frac{Yp \times Ys}{(Yp - Ys) \times (1 - \frac{Ys}{Yp})} \quad (21)$$

Tyshchenko et al. (2020) [36]
where ISR – stress resistance index.

Rosielle A.A. et al. [60] suggested using the tolerance index (*TOL*) as the difference between the yield under irrigation and the yield under natural moisture conditions, as well as the mean yield (*MP*) as the arithmetic mean of the yield under stress and optimal conditions. Blum A. [16, 61] determined the drought resistance index (*DI*), which was generally accepted for determining genotypes that provide high yields both under stress and under optimal conditions. Fisher R.A. et al. [62] recommend using the stress susceptibility index (SSI) to determine the stability of plant productivity, which records yield values under optimal and stressful conditions. The Stress Susceptibility Index (*SSI*) is a good indicator for identifying high-yielding genotypes that are also highly resistant to stress. As a rule, a lower level of *SSI* indicates less variation in the yield of a variety under stress and under optimal conditions. Fernandez C.J. and Saba J. et al. [66] advise using the stress tolerance index (*STI*) for screening high-yielding genotypes under conditions of stress and its absence, and also recommend its use in breeding programs. Stable varieties have higher values of this index. Studying the yield of mung bean genotypes (*Vigna radiata* L.) in stressful and optimal environments, Fernandez C.J. classified them into four groups:

group A – varieties that have equally high productivity in both environments;

group B – varieties with high productivity only under optimal conditions;

group C – varieties with high yield under stress;

group D – varieties with low productivity in both environments.

To determine the susceptibility of varieties to stress due to different intensity of drought in different years, Fernandez C.J. [66] and Kristin A.S. et al. [67] suggested using the geometric mean productivity (*GMP*) of cultivars in both environments. In addition, Gavuzzi et al. [64], Bouslama M. et al. [63] and Choukan R. et al. [73] suggested using the yield index (*YI*), yield stability index (*YSI*) and yield reduction index (*YRI*), respectively.

While studying drought resistance indices of maize, Moghaddam A et al. [74] stated that a low tolerance index (*TOL*) does not necessarily mean a high yield of a variety under stress conditions, because the yield of a certain variety may be low under optimal conditions and show less reduction under stress, which leads to a decrease in *TOL* and, accordingly, this variety can be defined as resistant to drought. But Fernandez C.J. [66] believed that the *TOL* and *SSI* indices more reflect the drought resistance of the variety. According to Naeemi M. et al. [75] the use of the *SSI* index to determine varieties resistant to drought is a false direction. They believe that since the formula for calculating this index used the yield share of a certain variety under stress to optimal conditions, as well as the ratio of productivity under stressed to non-stressed conditions in all varieties, then two varieties with high or low yields in both environments can have the same *SSI* value. With regard to *MP*, the authors found that the use of an average yield index often leads to the selection of cultivars with high yields under optimal conditions that are less tolerant to stress. Malek-Shahi F et al. [76] presented *MP* as a suitable index for determining drought-tolerant varieties. Shirani Rad A.H. et al. [77], studying the susceptibility to stress in six varieties of winter rapeseed, believe that the *GMP*, *STI* and *MP* indices are the most appropriate indices for determining drought-resistant varieties. The same opinion is held by Sio-Se-Mardeh A. et al. [78], who give importance to the *GMP*, *STI* and *MP* indices as the most effective for identifying varieties with high yields both during drought and under optimal conditions.

In order to increase the effectiveness of the *STI* index, Farshadfar E. et al. [70] proposed modified stress resistance indices (M_1STI, M_2STI) that adjust for *STI*. For the screening of drought-resistant genotypes in different environmental conditions, Moosavi S.S. et al. [69] presented the Stress Propensity Percentage Index (*SSPI*).

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