

The Study of Drought Stress in Sugar Beet and the Ways of its Minimization

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ABSTRACT

The article presents peculiarities of drought stress identification in sugar beet and the alternatives for the use of additional components of cultivation technology aimed at increasing tolerance to water deficiency at the early stages of growth and development, namely: (i) soil amendment with moisture-retaining polymers; (ii) seed treatment with a growth regulator contributing to a better formation of the root system; and (iii) foliar application of micro fertilizers. The study of the state of the plant photosynthetic apparatus was carried out with the use of the devices for measuring chlorophyll fluorescence FLORATEST, developed at the Institute of Cybernetics of the National Academy of Sciences of Ukraine. The measurements were performed according to the guidelines on Determination of the Fluorescence Induction of plant Chlorophyll: Theoretical and Practical Bases of the Method. For better representativeness of the sampling, the measurements were performed at the same time of day and with the same intensity of illumination of the plants. As a result of the studies, the ratio of variable to maximum fluorescence (F_v/F_m) of the plant photosystem obtained with the use of a portable fluorometer was found to be the most effective method of rapid diagnostics of drought stress in plants. A high level of correlation was found between the concentration of free proline and the F_v/F_m ratio, with the correlation coefficient for sugar beet $r = -0.96$, which corresponds to a very strong relationship.

Keywords: chlorophyll fluorescence, moisture-retaining polymers, growth regulator, microfertilizer, proline.

INTRODUCTION

Sugar beet demonstrates high potential productivity under the application of advanced cultivation technologies and the introduction of high-yielding hybrids. Thus, in Germany and France, it is realistic to obtain yields at the level of 110–150 t/ha and in Ukraine 90–110 t/ha (Tsvei et al, 2020). However, in Ukraine, in 2020, the average root yield was only 40.8 t/ha, which is less than half of the crop yield potential (FAO STAT, 2020). Vegetation conditions, such as air and soil temperature, precipitation, available soil

moisture, nutrients, etc. are major limiting factors of sugar beet performance. While the provision of high-quality nutrition and other agrotechnical measures can be controlled by producers, moisture and heat conditions depend on the conditions of the growing region. In the context of global climate changes, local climatic conditions also change, which negatively affects crop performance (Martínez Quesada et al, 2003; Milford and Houghton, 1999; Schick, 2020). The most valuable methods of instrumental identification of plant conditions are those that are rapid. The use of fluorometers or chlorophyll sensors, which

in field conditions register changes in photosynthetic activity in plant leaves, is a method of rapid plant diagnosis. Chlorophyll sensors are optoelectronic devices for converting the fluorescence of the plant photosystem into an electrical signal with further processing (Tripathy et al, 1981; van Kooten and Snel, 1990; Vredenberg, 2004). The shape of the chlorophyll fluorescence induction curve (IFC) and the ratio between its main components are quite sensitive to changes occurring in the plant photosynthetic apparatus. In the process of crop growth and development, they can adapt to various environmental conditions; however, everything that goes beyond the adaptive capabilities of plants is reflected in the activity of the photosystem and, as a result, reflects in the Kautsky curve. The excess light energy absorbed by the photosystem must be effectively dissipated by non-photochemical methods. Otherwise, excess energy accumulated by the photosynthetic apparatus can lead to its destruction. Therefore,

in photosynthetic organs occur the processes of heat radiation and reradiation of a small but diagnostically important amount of energy of absorbed radiation in the red/infrared range with a wavelength of up to 800 nm occurs (Ripley et al, 2004; Schreiber, 2004; Sholes and Rolfe, 1996).

MATERIALS AND METHODS

Field experiments were carried out in the zone of unstable soil moisture at the Experimental Field of the Institute of Bioenergy Crops and Sugar Beet (50.023194, 30.173895) in the years 2014–2018. The experimental design is shown in Table 1. Moisture-retaining polymers Aquasorb were introduced under early spring tillage using an Amazone ZA-TS 3200 spreader. The soil was treated with Mirazonit bacteria concentrate at a dose of 20 l/ha before cultivation using field mounted sprayer of the Amazone UF type at a working

Table 1. Scheme of the experiment on lentils and pea to study the factors of increasing tolerance to water deficiency at early stages of growth and development

Moisture-retaining agent	Soil amendment	Growth regulator	Micro fertilizers	Treatment
Control	Control	Control	Control	1
			Alpha-Grow-Extra Beets, 3 l/ha (BBCH 18)	2
			Micro-Mineralis (Beets), 1.5 l/ha (BBCH 18)	3
		Kelpak SC (2 l/ha) (BBCH 14) + 4 l/ha (BBCH 18)	Control	4
			Alpha-Grow-Extra Beets, 3 l/ha (BBCH 18)	5
			Micro-Mineralis (Beets), 1.5 l/ha (BBCH 18)	6
	Soil bacteria concentrate Mirazonit (20 l/ha)	Control	Control	7
			Alpha-Grow-Extra Beets, 3 l/ha (BBCH 18)	8
			Micro-Mineralis (Beets), 1.5 l/ha (BBCH 18)	9
		Kelpak SC (2 l/ha) (BBCH 14) + 4 l/ha (BBCH 18)	Control	10
			Alpha-Grow-Extra Beets, 3 l/ha (BBCH 18)	11
			Micro-Mineralis (Beets), 1.5 l/ha (BBCH 18)	12
Moisture-retaining polymers Aquasorb (300 kg/ha)	Control	Control	Control	13
			Alpha-Grow-Extra Beets, 3 l/ha (BBCH 18)	14
			Micro-Mineralis (Beets), 1.5 l/ha (BBCH 18)	15
		Kelpak SC (2 l/ha) (BBCH 14) + 4 l/ha (BBCH 18)	Control	16
			Alpha-Grow-Extra Beets, 3 l/ha (BBCH 18)	17
			Micro-Mineralis (Beets), 1.5 l/ha (BBCH 18)	18
	Soil bacteria concentrate Mirazonit (20 l/ha)	Control	Control	19
			Alpha-Grow-Extra Beets, 3 l/ha (BBCH 18)	20
			Micro-Mineralis (Beets), 1.5 l/ha (BBCH 18)	21
		Kelpak SC (2 l/ha) (BBCH 14) + 4 l/ha (BBCH 18)	Control	22
			Alpha-Grow-Extra Beets, 3 l/ha (BBCH 18)	23
			Micro-Mineralis (Beets), 1.5 l/ha (BBCH 18)	24

Note: The single plot area 50 m² and 35 m², 4 replications.

fluid flow rate of 200 l/ha. In our research, we used the Floratest device (Fig.1) developed at the V. M. Hlushkov Institute of Cybernetics of the National Academy of Sciences of Ukraine. Its authors are V. O. Romanov, Yu. O. Braiko, D. M. Artemenko (algorithm and hardware), R. G. Imamutdinova (software), V. S. Fedak (application methods) (Prsyazhniuk et al, 2016). The basic principles of the device operation are based on algorithms identical to foreign analogs, which ensure the determination of chlorophyll fluorescence intensity and exposure to radiation (Fig. 2).

The soil of the experimental field was deep medium-loam chernozem on loess loam with the following characteristics: humus content (by Tyurin) of 2.58%, alkaline hydrolyzed nitrogen (by Kornfeld) 176 mg/kg, mobile compounds of phosphorus and potassium (by Chirikov) 160 and 95 mg/kg, saline pH 6.75, the amount of absorbed alkali 305 mg-eq/kg, and hydrolytic acidity 9.1 mg-eq/kg. The content of humus and alkaline hydrolyzed nitrogen was average, the content of mobile phosphorus high, and the content of exchange potassium was high. The soil was composed of clay (37%) and sand (63%), with a density from 1.16 to 1.25 g/cm³, the humidity of permanent wilting of 10.8%, and the groundwater table level of 5–10 m. Weather conditions during the years of research varied from moderate moisture provision to severe drought, which corresponds to the zone of insufficient moisture according to the dynamics of weather parameters. The highest sugar beet plant need in moisture is observed in the period from the closing of the leaves in the row to the closing of the leaves in the interrow. In the row closure stage (BBCH 30), the lowest moisture reserves were in the 0–50 cm soil layer in 2017, and the highest in 2014. The rest of the years had close to average moisture availability indicators. The use of moisture-retaining polymers Aquasorb ensures an additional 5 mm of moisture available to plants during the growing season in the soil layer of 0–50 cm.

General and special agronomic methods were used to carry out field experiments (Prsyazhniuk et al, 2021). The measurement of plant biometrics was carried out by sampling 50 plants per replication. The content of free proline was determined by colorimetric analysis using ninhydrin (Carrillo et al., 2008; Carillo, Gibon, 2011). Statistical analysis was performed using the ANOVA method using the Statistica 12 software (Ermantraut et al, 2007).

RESULTS AND DISCUSSION

Among all indicators of the state of the plant photosystem, we chose the ratio of variable to maximum fluorescence F_v/F_m as an effective indicator of plant stress caused by a lack of moisture in the soil (drought stress). The F_v/F_m data of the sugar beet photosystem in the stage of the closing



Fig. 1. Measurement of chlorophyll fluorescence induction with Floratest

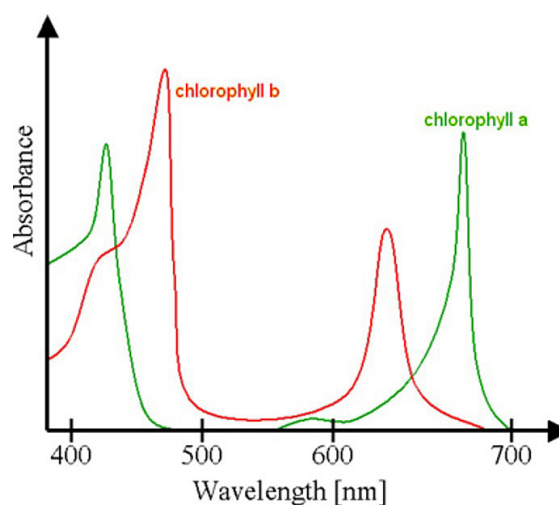


Fig. 2. The main spectra of absorption of light energy by chlorophylls, which are the base of Floratest operation

row under the applied agronomic practices are shown in Table 2.

Indicators of the ratio of variable to maximum fluorescence (F_v/F_m) in sugar beet varied as affected by the conditions of a vegetation season. Thus, lower F_v/F_m values were observed in the control treatments in the years with not sufficient soil moisture available for plants in the stage of closing the rows (BBCH 31). On average over the years of research, the F_v/F_m ratio was 0.41 when sugar beet plants in the stage of row closing (BBCH 31) experienced drought stress (control treatment). The application of a moisture-retaining agent significantly improved the physiological state of the plant photosystem, and the average F_v/F_m indicator for such variants of the experiment was 0.51, while without hydrogel it was 0.44. Moreover, additional agronomic practices, such as the introduction of moisture-retaining polymers AQUASORB (300 kg/ha), application of soil bacteria Mirazonit (20 l/ha) and growth regulator KELPAK SC (2 l/ha at BBCH

14 and 4 l/ha at BBCH 18) ensured stable work of the photosynthetic apparatus of plants. Foliar application of microfertilizer Alpha-Grow-Extra Beetroot, 3 l/ha (BBCH 18) or Micro-Mineralis (Beetroot), 1.5 l/ha (BBCH 18), on the contrary, turned out to be ineffective. The obtained differences normally were within the experimental error or absent, compared to the corresponding control treatments. This additionally confirms the minimal impact of microfertilizers in sugar beet cultivation. Many researchers have proven that the determination of chlorophyll fluorescence is a very sensitive method for identifying the physiological state of leaves and plant productivity in a wide range of conditions (Baker, 2008). Many studies revealed that the initial phase of stress in plants is especially important. After all, a further limitation of photosynthesis does not depend on the parameters of chlorophyll fluorescence (Cornic and Fresneau, 2002; Anjum et al., 2011; Cavender-Bares and Fakhri, 2004; Iannucci et al., 2000). The obtained equation and a graphical

Table 2. F_v/F_m index of sugar beet photosystem in the stage of closing rows (BBCH 31)

Treatment	2014	2015	2016	2017	2018
1	0.45	0.41	0.37	0.35	0.48
2	0.47	0.43	0.39	0.37	0.51
3	0.46	0.43	0.39	0.37	0.51
4	0.48	0.43	0.41	0.38	0.51
5	0.49	0.44	0.41	0.39	0.52
6	0.49	0.44	0.41	0.39	0.52
7	0.48	0.43	0.39	0.37	0.51
8	0.48	0.44	0.40	0.37	0.52
9	0.48	0.44	0.40	0.37	0.52
10	0.49	0.44	0.41	0.39	0.52
11	0.49	0.45	0.42	0.40	0.53
12	0.49	0.45	0.42	0.40	0.52
13	0.52	0.49	0.45	0.43	0.56
14	0.53	0.50	0.46	0.43	0.56
15	0.53	0.50	0.45	0.43	0.56
16	0.54	0.50	0.48	0.45	0.56
17	0.55	0.51	0.49	0.47	0.57
18	0.55	0.51	0.49	0.47	0.57
19	0.53	0.50	0.46	0.44	0.56
20	0.54	0.51	0.47	0.44	0.57
21	0.54	0.50	0.47	0.44	0.57
22	0.55	0.52	0.51	0.48	0.58
23	0.56	0.53	0.51	0.49	0.59
24	0.57	0.54	0.52	0.49	0.59
LSD _{0.05}	0.03	0.02	0.02	0.03	0.03

representation of the dependence between sugar beet yield and the F_v/F_m ratio of the photosystem are shown in Fig. 3.

The analysis of experimental data shows that there is a strong correlation between the yield of sugar beet and the indicators of the photosystem in the stage of row closing BBCH 31 ($r = 0.98$). Therefore, the most effective means of increasing the efficiency of the sugar beet photosystem was the use of moisture-retaining polymers AQUASORB (300 kg/ha), while other factors of the experiment little affected the F_v/F_m ratio. Proline belongs to the so-called ‘stress’ amino acids; therefore, the activation of its synthesis is observed not only during the aging of the plant organism but also during the development of a stress reaction. Therefore, the accumulation of proline in plant organs is considered an adaptive response of the plant to stress conditions (Kolupaev et al., 2014). Although proline is the most common metabolite that accumulates under stressful conditions (Ain-Lhout et al., 2001; Al-Khayri, 2002; Delauney and Verma, 1993), the significance of this accumulation in osmotic adjustment in plants is still debated, and different plant species’ response features are observed (Hoai and Shim, 2003). Thus, there is experimental evidence that proline accumulation is a symptom of trauma-induced stress rather than an indicator of stress tolerance (Hoai and Shim, 1997). At the same time, the dependence between the level of stress and the concentration of proline indicates that the accumulation of proline is an indicator that can be used to determine stress in sugar beet (Ain-Lhout et al., 2001; Iannucci et al., 2000; Qi et al.,

2005). Thus, proline acts as a signaling molecule to modulate mitochondrial functions, affect cell proliferation, and initiate specific gene expression, which may be important for plant recovery after stress (Al-Khayri, 2002; Szabados and Savoure, 2009; Valliyodan and Nguyen, 2006; Yamada et al., 2005). There are several studies examining the effect of environmental stress on the growth of sugar beet, conducted under controlled conditions and in the field (Kenter et al., 2006; Luković et al., 2009; Mezei et al., 2006).

It was found that in the control treatments (without the application of additional cultivation practices) plants were sensitive to drought in the critical periods of their growth and development as the maximum concentration of proline was observed in those periods. However, treatment with the moisture-retaining agent helped to alleviate stress in plants and minimize the content of free proline in their photosynthetic organs. Similar studies conducted by other scientists are consistent with our results. Thus, in wheat, the proline content gradually increased with the duration of stress, and a faster accumulation of proline was observed rather in the tolerant genotype than in the susceptible one (Nayyar and Walia, 2003; Song et al., 2005). In another study on corn, the proline content increased in the leaves of plants grown under conditions of water deficit compared to control plants (Anjum et al., 2011; Koskeroglu and Tuna, 2010). There are also many studies on various crops that confirm the relationship between the content of free proline and the effect of drought on plants (Bartels and Sunkar, 2005; Buschmann, 2010; Campbell, 2002; Chaves et

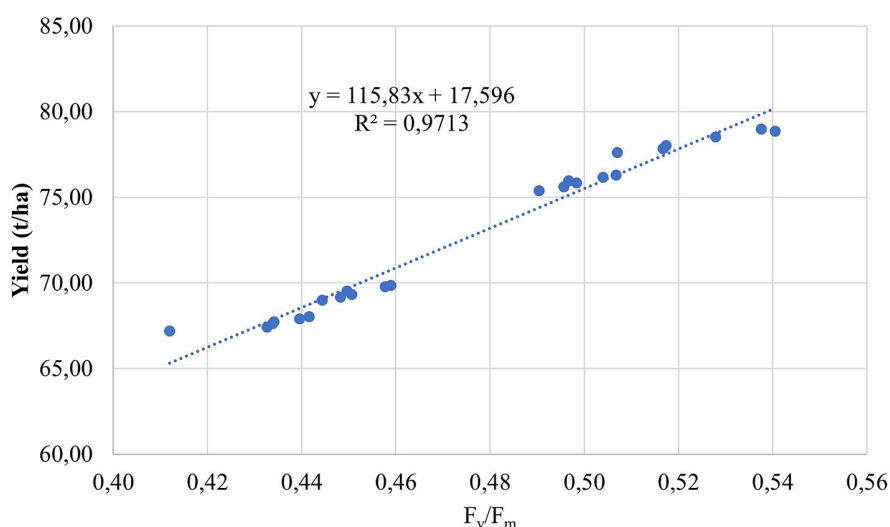


Fig. 3. Regression relationship between sugar beet productivity and the F_v/F_m ratio

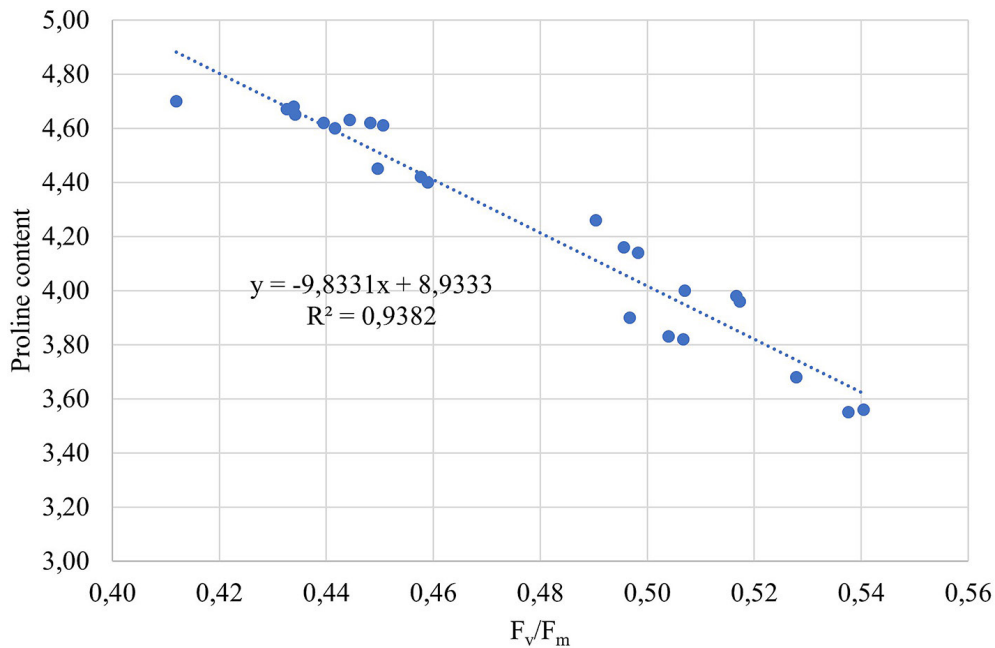


Fig. 4. Regression relationship between the content of proline in sugar beet and the F_v/F_m indicator of the photosystem

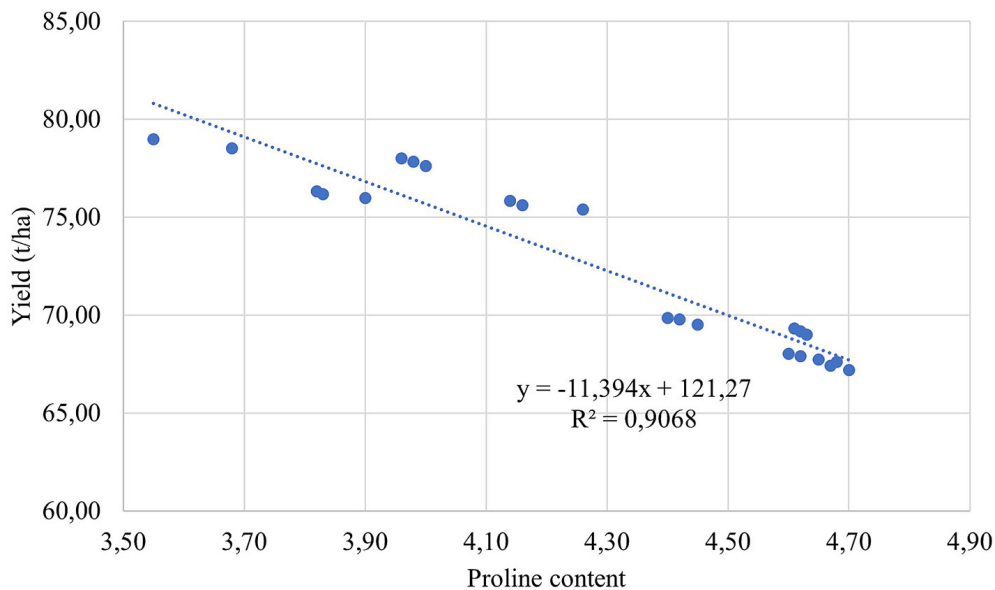


Fig. 5. Regression relationship between proline content in sugar beet and root yield

al., 2009; Coca et al., 2004; Conde et al., 2011; Monreal et al., 2007; Qin et al., 2011; Roy et al., 2009). An important issue is the determination of the characteristics (type and strength) of the dependence between the content of free proline and the F_v/F_m ratio of the sugar beet photosystem (Fig. 4).

The research results point out a possibility of a strong correlation between the concentration of free proline and the ratio F_v/F_m , as evidenced by the correlation coefficient $r = -0.96$. We also determined the regression relationships between

the proline content in the studied crops and their yield (Fig. 5). The obtained regularities point out a high concentration of free proline as a reliable indicator of plant stress caused by drought conditions, which in turn relates to the level of crop productivity. Thus, we obtained a correlation coefficient for sugar beet $r = -0.95$, which corresponds to a very strong correlation. Moreover, free proline does not affect crop performance, it is only an indicator of the level of plant stress. The stress caused by the lack of moisture in the

soil negatively affects not only the processes of photosynthesis, but also plant growth and development and, consequently, crop productivity.

CONCLUSIONS

Analysis of the ratio of variable to maximum fluorescence (F_v/F_m) of plant photosystem was found the most effective method of rapid diagnosis of drought stress in plants with the use of portable fluorimeters. Our results showed a high correlation between the concentration of free proline and the ratio of variable to maximum fluorescence (F_v/F_m) of plant photosystem, with the correlation coefficient for sugar beet $r = -0.96$, which corresponds to a very strong correlation.

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